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Search Results - Record(s) 1 through 10 of 11 returned.

☐ 1. Document ID: US 20020150996 A1

L11: Entry 1 of 11 File: PGPB Oct 17, 2002

PGPUB-DOCUMENT-NUMBER: 20020150996
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020150996 A1

TITLE: Functional nucleic acid probes and uses thereof

PUBLICATION-DATE: October 17, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Nilsen-Hamilton, Marit	Ames	IA	US	

US-CL-CURRENT: 435/91.2; 435/455, 514/44

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 2. Document ID: US 20020102568 A1

L11: Entry 2 of 11 File: PGPB Aug 1, 2002

PGPUB-DOCUMENT-NUMBER: 20020102568
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020102568 A1

TITLE: Nucleic acid sensor molecules

PUBLICATION-DATE: August 1, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Usman, Nassim	Lafayette	CO	US	
McSwiggen, James A.	Boulder	CO	US	
Zinnen, Shawn	Denver	CO	US	
Seiwert, Scott	Lyons	CO	US	
Haeberli, Peter	Berthoud	CO	US	
Chowrira, Bharat	Broomfield	CO	US	
Blatt, Lawrence	Boulder	CO	US	
Vaish, Narendra K.	Boulder	CO	US	

US-CL-CURRENT: 435/6; 536/24.3

[redacted]

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 3. Document ID: US 20020034757 A1

L11: Entry 3 of 11 File: PGPB Mar 21, 2002

PGPUB-DOCUMENT-NUMBER: 20020034757
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020034757 A1

TITLE: Single-molecule selection methods and compositions therefrom

PUBLICATION-DATE: March 21, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Cubicciotti, Roger S.	Montclair	NJ	US	

US-CL-CURRENT: 435/6; 435/91.2, 536/22.1, 536/23.1, 536/24.3

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 4. Document ID: US 20020028457 A1

L11: Entry 4 of 11 File: PGPB Mar 7, 2002

PGPUB-DOCUMENT-NUMBER: 20020028457
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020028457 A1

TITLE: Single target counting assays using semiconductor nanocrystals

PUBLICATION-DATE: March 7, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Empedocles, Stephen Alexander	Mountain View	CA	US	
Watson, Andrew R.	Belmont	CA	US	
Phillips, Vince	Sunnyvale	CA	US	
Wong, Edith	Danville	CA	US	

US-CL-CURRENT: 435/6; 435/8

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 5. Document ID: US 20010055764 A1

L11: Entry 5 of 11 File: PGPB Dec 27, 2001

[redacted]

PGPUB-DOCUMENT-NUMBER: 20010055764
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20010055764 A1

TITLE: Microarray methods utilizing semiconductor nanocrystals

PUBLICATION-DATE: December 27, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Empedocles, Stephen A.	Mountain View	CA	US	
Wong, Edith Y.	Danville	CA	US	
Phillips, Vince E.	Sunnyvale	CA	US	
Daniels, R. Hugh	Palo Alto	CA	US	

US-CL-CURRENT: 435/6; 435/7.92

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 6. Document ID: US 20010024789 A1

L11: Entry 6 of 11 File: PGPB Sep 27, 2001

PGPUB-DOCUMENT-NUMBER: 20010024789
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20010024789 A1

TITLE: Methods for generating catalytic proteins

PUBLICATION-DATE: September 27, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Kurz, Markus	West Newton	MA	US	
Lohse, Peter	Weston	MA	US	

US-CL-CURRENT: 435/6; 435/69.1, 435/7.92

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 7. Document ID: US 6387703 B1

L11: Entry 7 of 11 File: USPT May 14, 2002

US-PAT-NO: 6387703
DOCUMENT-IDENTIFIER: US 6387703 B1

TITLE: Method for modulating gene expression

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 8. Document ID: US 6287765 B1

L11: Entry 8 of 11 File: USPT Sep 11, 2001

US-PAT-NO: 6287765
DOCUMENT-IDENTIFIER: US 6287765 B1

TITLE: Methods for detecting and identifying single molecules

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 9. Document ID: US 5851786 A

L11: Entry 9 of 11 File: USPT Dec 22, 1998

US-PAT-NO: 5851786
DOCUMENT-IDENTIFIER: US 5851786 A

TITLE: Product and process to regulate actin polymerization

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 10. Document ID: AU 200235737 A WO 200242491 A2 DE 10057853 A1

L11: Entry 10 of 11 File: DWPI Jun 3, 2002

DERWENT-ACC-NO: 2002-471731
DERWENT-WEEK: 200263

TITLE: Identifying agents that inhibit the function of specific RNA, useful e.g. for treating human or feline immune deficiency virus, comprises using a target-reporter construct prepared from a reporter ribozyme domain

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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Term	Documents
ALLOSTERIC.DWPI,EPAB,JPAB,USPT,PGPB.	1907
ALLOSTERICS.DWPI,EPAB,JPAB,USPT,PGPB.	2
RIBOZYME.DWPI,EPAB,JPAB,USPT,PGPB.	6961
RIBOZYMES.DWPI,EPAB,JPAB,USPT,PGPB.	6590
((ALLOSTERIC SAME RIBOZYME) NOT 10).USPT,PGPB,JPAB,EPAB,DWPI.	11
(ALLOSTERIC SAME RIBOZYME NOT L10).USPT,PGPB,JPAB,EPAB,DWPI.	11

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Search Results - Record(s) 1 through 10 of 36 returned.

☐ 1. Document ID: US 20020182612 A1

L9: Entry 1 of 36 File: PGPB Dec 5, 2002

PGPUB-DOCUMENT-NUMBER: 20020182612
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020182612 A1

TITLE: Therapeutics and diagnostics based on a novel IL-1beta mutation

PUBLICATION-DATE: December 5, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Duff, Gordon W.	Sheffield		GB	
Giovine, Francesco Saverio di	Ranmoor		GB	

US-CL-CURRENT: 435/6; 435/91.2, 536/24.3

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWMC | Draw Desc | Image

☐ 2. Document ID: US 20020168338 A1

L9: Entry 2 of 36 File: PGPB Nov 14, 2002

PGPUB-DOCUMENT-NUMBER: 20020168338
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020168338 A1

TITLE: COMPOSITIONS AND METHODS FOR DELIVERY OF AGENTS FOR NEURONAL REGENERATION AND SURVIVAL

PUBLICATION-DATE: November 14, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
BAIRD, ANDREW			US	

US-CL-CURRENT: 424/93.2; 424/193.1, 424/423, 424/424, 424/425, 424/468, 424/469, 424/486, 435/320.1, 514/44, 536/24.1, 536/24.5

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWMC | Draw Desc | Image

☐ 3. Document ID: US 20020142305 A1

L9: Entry 3 of 36 File: PGPB Oct 3, 2002

PGPUB-DOCUMENT-NUMBER: 20020142305
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020142305 A1

TITLE: Methods for diagnosing and monitoring ovarian cancer by screening gene copy numbers

PUBLICATION-DATE: October 3, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Chin, Koei	Foster City	CA	US	
Kuo, Wen-Lin	Pleasanton	CA	US	
Pinkel, Daniel	Walnut Creek	CA	US	
Albertson, Donna	Lafayette	CA	US	
Collins, Colin	San Raphael	CA	US	
Gray, Joe W.	San Francisco	CA	US	

US-CL-CURRENT: 435/6

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 4. Document ID: US 20020107218 A1

L9: Entry 4 of 36 File: PGPB Aug 8, 2002

PGPUB-DOCUMENT-NUMBER: 20020107218
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020107218 A1

TITLE: Inhibition of stress activated protein kinase (SAPK) pathway and sensitization of cells to cancer therapies

PUBLICATION-DATE: August 8, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Mercola, Daniel	Rancho Santa Fe	CA	US	

US-CL-CURRENT: 514/44; 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 5. Document ID: US 20020106647 A1

L9: Entry 5 of 36 File: PGPB Aug 8, 2002

PGPUB-DOCUMENT-NUMBER: 20020106647

PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020106647 A1

TITLE: Nucleic acid compositions and methods of introducing nucleic acids into cells

PUBLICATION-DATE: August 8, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Segal, Andrew H.	Cambridge	MA	US	
Wilson, Jeffrey	Brighton	MA	US	

US-CL-CURRENT: 435/6; 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 6. Document ID: US 20020090643 A1

L9: Entry 6 of 36 File: PGPB Jul 11, 2002

PGPUB-DOCUMENT-NUMBER: 20020090643
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020090643 A1

TITLE: COMPOSITIONS AND METHODS FOR MONITORING THE PHOSPHORYLATION OF NATURAL BINDING PARTNERS

PUBLICATION-DATE: July 11, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
CRAIG, ROGER K.	CHESHIRE		GB	
COYLER, JOHN	WEST YORKSHIRE		GB	

US-CL-CURRENT: 435/7.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 7. Document ID: US 20020068354 A1

L9: Entry 7 of 36 File: PGPB Jun 6, 2002

PGPUB-DOCUMENT-NUMBER: 20020068354
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020068354 A1

TITLE: Feline immunodeficiency virus gene therapy vectors

PUBLICATION-DATE: June 6, 2002

INVENTOR-INFORMATION:

[redacted]

NAME	CITY	STATE	COUNTRY	RULE-47
Johnston, Julie C.	Hockessin	DE	US	
Sauter, Sybille L.	Del Mar	CA	US	
Hsu, David Chi-Tang	San Diego	CA	US	
Sheridan, Philip Lee	San Diego	CA	US	
Hardy, Stephen F.	San Francisco	CA	US	
Dubensky, Thomas W. JR.	Piedmont	CA	US	
Yee, Jiing-Kuan	Arcadia	CA	US	

US-CL-CURRENT: 435/235.1; 435/320.1, 435/456, 530/826, 536/23.72, 536/24.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 8. Document ID: US 20020052037 A1

L9: Entry 8 of 36 File: PGPB May 2, 2002

PGPUB-DOCUMENT-NUMBER: 20020052037
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020052037 A1

TITLE: Nucleic acid catalysts comprising L-nucleotide analogs

PUBLICATION-DATE: May 2, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Beigelman, Leonid	Longmont	CO	US	

US-CL-CURRENT: 435/199; 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 9. Document ID: US 20020048805 A1

L9: Entry 9 of 36 File: PGPB Apr 25, 2002

PGPUB-DOCUMENT-NUMBER: 20020048805
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020048805 A1

TITLE: FELINE IMMUNODEFICIENCY VIRUS GENE THERAPY VECTORS

PUBLICATION-DATE: April 25, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
JOHNSTON, JULIE C.	WILMINGTON	DE	US	
SAUTER, SYBILLE L.	DEL MAR	CA	US	
HSU, DAVID CHI-TANG	SAN DIEGO	CA	US	
SHERIDAN, PHILIP LEE	SAN DIEGO	CA	US	
HARDY, STEPHEN F.	SAN FRANCISCO	CA	US	
DUBENSKY, THOMAS W. JR.	PIEDMONT	CA	US	
YEE, JIING-KUAN	DEL MAR	CA	US	

US-CL-CURRENT: 435/235.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 10. Document ID: US 20020028919 A1

L9: Entry 10 of 36 File: PGPB Mar 7, 2002

PGPUB-DOCUMENT-NUMBER: 20020028919
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020028919 A1

TITLE: Xylofuranosly-containing nucleoside phosphoramidites and polynucleotides

PUBLICATION-DATE: March 7, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Matulic-Adamic, Jasenka	Boulder	CO	US	
Beigelman, Leonid	Longmont	CO	US	

US-CL-CURRENT: 536/5; 530/322, 536/23.1, 536/26.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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Term	Documents
TARGET.DWPI,EPAB,JPAB,USPT,PGPB.	436210
TARGETS.DWPI,EPAB,JPAB,USPT,PGPB.	68803
DELIVER.DWPI,EPAB,JPAB,USPT,PGPB.	234538
DELIVERS.DWPI,EPAB,JPAB,USPT,PGPB.	143429
BIND$	0
BIND.DWPI,EPAB,JPAB,USPT,PGPB.	203725
BINDA.DWPI,EPAB,JPAB,USPT,PGPB.	479
BINDABILITIES.DWPI,EPAB,JPAB,USPT,PGPB.	2
BINDABILITY.DWPI,EPAB,JPAB,USPT,PGPB.	197
BINDABILITY-PROMOTING.DWPI,EPAB,JPAB,USPT,PGPB.	1
BINDABLE.DWPI,EPAB,JPAB,USPT,PGPB.	1088
(L8 AND (TARGET OR DELIVER OR BIND$) AND (LINKER$ OR BRIDG$)).USPT,PGPB,JPAB,EPAB,DWPI.	36

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Search Results - Record(s) 11 through 20 of 36 returned.

☐ 11. Document ID: US 20020019002 A1

L9: Entry 11 of 36 File: PGPB Feb 14, 2002

PGPUB-DOCUMENT-NUMBER: 20020019002
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020019002 A1

TITLE: Methods of monitoring enzyme activity

PUBLICATION-DATE: February 14, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Griffiths, Gary	Oldham		GB	

US-CL-CURRENT: 435/6

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 12. Document ID: US 20020007051 A1

L9: Entry 12 of 36 File: PGPB Jan 17, 2002

PGPUB-DOCUMENT-NUMBER: 20020007051
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020007051 A1

TITLE: Use of multiple recombination sites with unique specificity in recombinational cloning

PUBLICATION-DATE: January 17, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Cheo, David	Kensington	MD	US	
Brasch, Michael A.	Gaithersburg	MD	US	
Temple, Gary F.	Washington Grove	MD	US	
Hartley, James L.	Frederick	MD	US	
Byrd, Devon R. N.	Montgomery Village	MD	US	

US-CL-CURRENT: 536/23.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 13. Document ID: US 6489465 B2

L9: Entry 13 of 36 File: USPT Dec 3, 2002

US-PAT-NO: 6489465
DOCUMENT-IDENTIFIER: US 6489465 B2

TITLE: Xylofuranosly-containing nucleoside phosphoramidites and polynucleotides

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 14. Document ID: US 6482803 B1

L9: Entry 14 of 36 File: USPT Nov 19, 2002

US-PAT-NO: 6482803
DOCUMENT-IDENTIFIER: US 6482803 B1

TITLE: Modification of mutated P53 gene in tumors by retroviral delivery of ribozyme A

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 15. Document ID: US 6465199 B1

L9: Entry 15 of 36 File: USPT Oct 15, 2002

US-PAT-NO: 6465199
DOCUMENT-IDENTIFIER: US 6465199 B1

TITLE: Compositions and methods for monitoring the modification of natural binding partners

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 16. Document ID: US 6458559 B1

L9: Entry 16 of 36 File: USPT Oct 1, 2002

US-PAT-NO: 6458559
DOCUMENT-IDENTIFIER: US 6458559 B1

TITLE: Multivalent RNA aptamers and their expression in multicellular organisms

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 17. Document ID: US 6387617 B1

L9: Entry 17 of 36 File: USPT May 14, 2002

US-PAT-NO: 6387617
DOCUMENT-IDENTIFIER: US 6387617 B1

TITLE: Catalytic nucleic acid and methods of use

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 18. Document ID: US 6379931 B1

L9: Entry 18 of 36 File: USPT Apr 30, 2002

US-PAT-NO: 6379931
DOCUMENT-IDENTIFIER: US 6379931 B1

TITLE: Chimeric DNA/RNA ribozymes containing propanediol

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 19. Document ID: US 6316612 B1

L9: Entry 19 of 36 File: USPT Nov 13, 2001

US-PAT-NO: 6316612
DOCUMENT-IDENTIFIER: US 6316612 B1

TITLE: Xylofuranosly-containing nucleoside phosphoramidites and polynucleotides

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 20. Document ID: US 6251666 B1

L9: Entry 20 of 36 File: USPT Jun 26, 2001

US-PAT-NO: 6251666
DOCUMENT-IDENTIFIER: US 6251666 B1

TITLE: Nucleic acid catalysts comprising L-nucleotide analogs

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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Term	Documents
TARGET.DWPI,EPAB,JPAB,USPT,PGPB.	436210
TARGETS.DWPI,EPAB,JPAB,USPT,PGPB.	68803
DELIVER.DWPI,EPAB,JPAB,USPT,PGPB.	234538
DELIVERS.DWPI,EPAB,JPAB,USPT,PGPB.	143429
BIND$	0
BIND.DWPI,EPAB,JPAB,USPT,PGPB.	203725
BINDA.DWPI,EPAB,JPAB,USPT,PGPB.	479
BINDABILITIES.DWPI,EPAB,JPAB,USPT,PGPB.	2
BINDABILITY.DWPI,EPAB,JPAB,USPT,PGPB.	197
BINDABILITY-PROMOTING.DWPI,EPAB,JPAB,USPT,PGPB.	1
BINDABLE.DWPI,EPAB,JPAB,USPT,PGPB.	1088
(L8 AND (TARGET OR DELIVER OR BIND$) AND (LINKER$ OR BRIDG$)).USPT,PGPB,JPAB,EPAB,DWPI.	36

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Search Results - Record(s) 21 through 30 of 36 returned.

☐ 21. Document ID: US 6232064 B1

L9: Entry 21 of 36 File: USPT May 15, 2001

US-PAT-NO: 6232064
DOCUMENT-IDENTIFIER: US 6232064 B1

TITLE: Methods of diagnosing a pathology characterized by a cell proliferative disorder associated with connective tissue growth factor

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 22. Document ID: US 6197751 B1

L9: Entry 22 of 36 File: USPT Mar 6, 2001

US-PAT-NO: 6197751
DOCUMENT-IDENTIFIER: US 6197751 B1

TITLE: Thymosin .alpha.1 promotes tissue repair, angiogenesis and cell migration

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 23. Document ID: US 6194150 B1

L9: Entry 23 of 36 File: USPT Feb 27, 2001

US-PAT-NO: 6194150
DOCUMENT-IDENTIFIER: US 6194150 B1

TITLE: Nucleic acid based inhibition of CD40

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 24. Document ID: US 6159951 A

L9: Entry 24 of 36 File: USPT Dec 12, 2000

US-PAT-NO: 6159951
DOCUMENT-IDENTIFIER: US 6159951 A

TITLE: 2'-O-amino-containing nucleoside analogs and polynucleotides

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 25. Document ID: US 6150101 A

L9: Entry 25 of 36 File: USPT Nov 21, 2000

US-PAT-NO: 6150101
DOCUMENT-IDENTIFIER: US 6150101 A

TITLE: Methods of identifying a composition that alters connective tissue growth factor expression

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 26. Document ID: US 6103890 A

L9: Entry 26 of 36 File: USPT Aug 15, 2000

US-PAT-NO: 6103890
DOCUMENT-IDENTIFIER: US 6103890 A

TITLE: Enzymatic nucleic acids that cleave C-fos

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 27. Document ID: US 6069006 A

L9: Entry 27 of 36 File: USPT May 30, 2000

US-PAT-NO: 6069006
DOCUMENT-IDENTIFIER: US 6069006 A

TITLE: Connective tissue growth factor (CTGF) regulatory nucleic acid sequences

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 28. Document ID: US 6057156 A

L9: Entry 28 of 36 File: USPT May 2, 2000

US-PAT-NO: 6057156
DOCUMENT-IDENTIFIER: US 6057156 A

TITLE: Enzymatic nucleic acid treatment of diseases or conditions related to levels of epidermal growth factor receptors

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 29. Document ID: US 5877021 A

L9: Entry 29 of 36 File: USPT Mar 2, 1999

US-PAT-NO: 5877021
DOCUMENT-IDENTIFIER: US 5877021 A

TITLE: B7-1 targeted ribozymes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 30. Document ID: US 5817796 A

L9: Entry 30 of 36 File: USPT Oct 6, 1998

US-PAT-NO: 5817796
DOCUMENT-IDENTIFIER: US 5817796 A

TITLE: C-myb ribozymes having 2'-5'-linked adenylate residues

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

Generate Collection | Print

Term	Documents
TARGET.DWPI,EPAB,JPAB,USPT,PGPB.	436210
TARGETS.DWPI,EPAB,JPAB,USPT,PGPB.	68803
DELIVER.DWPI,EPAB,JPAB,USPT,PGPB.	234538
DELIVERS.DWPI,EPAB,JPAB,USPT,PGPB.	143429
BIND$	0
BIND.DWPI,EPAB,JPAB,USPT,PGPB.	203725
BINDA.DWPI,EPAB,JPAB,USPT,PGPB.	479
BINDABILITIES.DWPI,EPAB,JPAB,USPT,PGPB.	2
BINDABILITY.DWPI,EPAB,JPAB,USPT,PGPB.	197
BINDABILITY-PROMOTING.DWPI,EPAB,JPAB,USPT,PGPB.	1
BINDABLE.DWPI,EPAB,JPAB,USPT,PGPB.	1088
(L8 AND (TARGET OR DELIVER OR BIND$) AND (LINKER$ OR BRIDG$)).USPT,PGPB,JPAB,EPAB,DWPI.	36

There are more results than shown above. Click here to view the entire set.

[redacted]

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Search Results - Record(s) 31 through 36 of 36 returned.

☐ 31. Document ID: US 5770209 A

L9: Entry 31 of 36 File: USPT Jun 23, 1998

US-PAT-NO: 5770209
DOCUMENT-IDENTIFIER: US 5770209 A

TITLE: Acceleration of wound healing using connective tissue growth factor

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 32. Document ID: US 5763171 A

L9: Entry 32 of 36 File: USPT Jun 9, 1998

US-PAT-NO: 5763171
DOCUMENT-IDENTIFIER: US 5763171 A

TITLE: Nucleic acid structures with catalytic and autocatalytic replicating features and methods of use

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 33. Document ID: US 5688670 A

L9: Entry 33 of 36 File: USPT Nov 18, 1997

US-PAT-NO: 5688670
DOCUMENT-IDENTIFIER: US 5688670 A

TITLE: Self-modifying RNA molecules and methods of making

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 34. Document ID: US 5663064 A

L9: Entry 34 of 36 File: USPT Sep 2, 1997

US-PAT-NO: 5663064
DOCUMENT-IDENTIFIER: US 5663064 A

[redacted]

TITLE: Ribozymes with RNA protein binding site

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 35. Document ID: US 5646042 A

L9: Entry 35 of 36 File: USPT Jul 8, 1997

US-PAT-NO: 5646042
DOCUMENT-IDENTIFIER: US 5646042 A

TITLE: C-myb targeted ribozymes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 36. Document ID: US 5472840 A

L9: Entry 36 of 36 File: USPT Dec 5, 1995

US-PAT-NO: 5472840
DOCUMENT-IDENTIFIER: US 5472840 A

TITLE: Nucleic acid structures with catalytic and autocatalytic replicating features and methods of use

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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Term	Documents
TARGET.DWPI,EPAB,JPAB,USPT,PGPB.	436210
TARGETS.DWPI,EPAB,JPAB,USPT,PGPB.	68803
DELIVER.DWPI,EPAB,JPAB,USPT,PGPB.	234538
DELIVERS.DWPI,EPAB,JPAB,USPT,PGPB.	143429
BIND$	0
BIND.DWPI,EPAB,JPAB,USPT,PGPB.	203725
BINDA.DWPI,EPAB,JPAB,USPT,PGPB.	479
BINDABILITIES.DWPI,EPAB,JPAB,USPT,PGPB.	2
BINDABILITY.DWPI,EPAB,JPAB,USPT,PGPB.	197
BINDABILITY-PROMOTING.DWPI,EPAB,JPAB,USPT,PGPB.	1
BINDABLE.DWPI,EPAB,JPAB,USPT,PGPB.	1088
(L8 AND (TARGET OR DELIVER OR BIND$) AND (LINKER$ OR BRIDG$)).USPT,PGPB,JPAB,EPAB,DWPI.	36

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Search Results -

Term	Documents
BRIDG$	0
BRIDG.DWPI,EPAB,JPAB,USPT,PGPB.	49
BRIDGA.DWPI,EPAB,JPAB,USPT,PGPB.	1
BRIDGABILITY.DWPI,EPAB,JPAB,USPT,PGPB.	7
BRIDGABLE.DWPI,EPAB,JPAB,USPT,PGPB.	32
BRIDGADE.DWPI,EPAB,JPAB,USPT,PGPB.	4
BRIDGALL.DWPI,EPAB,JPAB,USPT,PGPB.	11
BRIDGALL-ET-AL.DWPI,EPAB,JPAB,USPT,PGPB.	11
BRIDGAM.DWPI,EPAB,JPAB,USPT,PGPB.	1
BRIDGAMAN.DWPI,EPAB,JPAB,USPT,PGPB.	1
BRIDGBON.DWPI,EPAB,JPAB,USPT,PGPB.	1
(L4 AND BRIDG$).USPT,PGPB,JPAB,EPAB,DWPI.	79

There are more results than shown above. Click here to view the entire set.

Database: US Patents Full-Text Database; US Pre-Grant Publication Full-Text Database; JPO Abstracts Database; EPO Abstracts Database; Derwent World Patents Index; IBM Technical Disclosure Bulletins

Search: L5

Refine Search

Recall Text | Clear

Search History

DATE: Saturday, December 21, 2002 Printable Copy Create Case

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L5	L4 and bridg$	79	L5
L4	L3 and domain	135	L4
L3	L2 and hammerhead	153	L3
L2	ribozyme same ligand	594	L2
DB=USPT; PLUR=YES; OP=ADJ			
L1	ribozyme same ligand	257	L1

END OF SEARCH HISTORY

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Generate Collection | Print

Search Results - Record(s) 1 through 10 of 79 returned.

☐ 1. Document ID: US 20020192749 A1

L5: Entry 1 of 79 File: PGPB Dec 19, 2002

PGPUB-DOCUMENT-NUMBER: 20020192749
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020192749 A1

TITLE: Human polynucleotides, polypeptides, and antibodies

PUBLICATION-DATE: December 19, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Moore, Paul A.	Germantown	MD	US	
Coleman, Timothy A.	Gaithersburg	MD	US	
Gentz, Reiner L.	Rockville	MD	US	
Dillon, Patrick J.	Carlsbad	CA	US	
Ni, Jian	Germantown	MD	US	
Li, Yi	Sunnyvale	CA	US	
Endress, Gregory A.	Florence	MA	US	
Soppet, Daniel R.	Centreville	VA	US	

US-CL-CURRENT: 435/69.1; 435/183, 435/320.1, 435/325, 530/350, 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

☐ 2. Document ID: US 20020187526 A1

L5: Entry 2 of 79 File: PGPB Dec 12, 2002

PGPUB-DOCUMENT-NUMBER: 20020187526
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020187526 A1

TITLE: Neutrokine-alpha binding proteins and methods based thereon

PUBLICATION-DATE: December 12, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Ruben, Steven M.	Olney	MD	US	
Ullrich, Stephen	Rockville	MD	US	
Baker, Kevin	Darnestown	MD	US	

US-CL-CURRENT: 435/69.5; 435/320.1, 435/325, 530/351, 536/23.5

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC
Draw Desc | Image

☐ 3. Document ID: US 20020183503 A1

L5: Entry 3 of 79 File: PGPB Dec 5, 2002

PGPUB-DOCUMENT-NUMBER: 20020183503
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020183503 A1

TITLE: 26 human secreted proteins

PUBLICATION-DATE: December 5, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Ruben, Steven M.	Olney	MD	US	
Birse, Charles E.	North Potomac	MD	US	
Duan, Roxanne D.	Bethesda	MD	US	
Soppet, Daniel R.	Laytonsville	MD	US	
Rosen, Craig A.	Laytonsville	MD	US	
Shi, Yanggu	Gaithersburg	MD	US	
LaFleur, David W.	Washington	DC	US	
Olsen, Henrik	Gaithersburg	MD	US	
Ebner, Reinhard	Gaithersburg	MD	US	
Florence, Kimberly A.	Rockville	MD	US	
Ni, Jian	Germantown	MD	US	
Young, Paul	Gaithersburg	MD	US	

US-CL-CURRENT: 536/23.2; 435/183, 435/320.1, 435/325, 435/6, 435/69.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 4. Document ID: US 20020168729 A1

L5: Entry 4 of 79 File: PGPB Nov 14, 2002

PGPUB-DOCUMENT-NUMBER: 20020168729
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020168729 A1

TITLE: Human endokine alpha and methods of use

PUBLICATION-DATE: November 14, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Yu, Guo-Liang	Berkeley	CA	US	
Ni, Jian	Rockville	MD	US	
Rosen, Craig A.	Laytonsville	MD	US	

US-CL-CURRENT: 435/69.5; 435/320.1, 435/325, 530/351, 536/23.5

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 5. Document ID: US 20020165377 A1

L5: Entry 5 of 79 File: PGPB Nov 7, 2002

PGPUB-DOCUMENT-NUMBER: 20020165377
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020165377 A1

TITLE: ADAM polynucleotides, polypeptides, and antibodies

PUBLICATION-DATE: November 7, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Ruben, Steven M.	Olney	MD	US	
Wei, Ping	Brookeville	MD	US	
Ni, Jian	Germantown	MD	US	
Hastings, Gregg A.	Westlake Village	CA	US	
Shi, Yanggu	Gaithersburg	MD	US	

US-CL-CURRENT: 536/23.2; 435/226, 435/320.1, 435/325, 435/69.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 6. Document ID: US 20020157122 A1

L5: Entry 6 of 79 File: PGPB Oct 24, 2002

PGPUB-DOCUMENT-NUMBER: 20020157122
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020157122 A1

TITLE: Beta-secretase transgenic organisms, anti-beta-secretase antibodies, and methods of use thereof

PUBLICATION-DATE: October 24, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Wong, Philip C.	Timonium	MD	US	
Cai, Huaibin	Baltimore	MD	US	
Price, Donald L.	Columbia	MD	US	

US-CL-CURRENT: 800/12; 424/146.1, 435/6, 435/7.1, 435/7.2, 514/44

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 7. Document ID: US 20020155564 A1

L5: Entry 7 of 79 File: PGPB Oct 24, 2002

PGPUB-DOCUMENT-NUMBER: 20020155564
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020155564 A1

TITLE: Cloning of a high growth gene

PUBLICATION-DATE: October 24, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Medrano, Juan F.	Davis	CA	US	
Bradford, Eric	Davis	CA	US	
Horvat, Simon	Edinburgh		GB	

US-CL-CURRENT: 435/184; 435/320.1, 435/325, 435/69.2, 536/23.2, 800/14

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 8. Document ID: US 20020152483 A1

L5: Entry 8 of 79 File: PGPB Oct 17, 2002

PGPUB-DOCUMENT-NUMBER: 20020152483
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020152483 A1

TITLE: Novel gene associated with regulation of adiposity and insulin response

PUBLICATION-DATE: October 17, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Reue, Karen	Torrance	CA	US	
Peterfy, Miklos	Los Angeles	CA	US	

US-CL-CURRENT: 800/9; 435/6, 435/91.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 9. Document ID: US 20020151004 A1

L5: Entry 9 of 79 File: PGPB Oct 17, 2002

PGPUB-DOCUMENT-NUMBER: 20020151004
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020151004 A1

TITLE: Delivery vehicles and methods for using the same

PUBLICATION-DATE: October 17, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Craig, Roger	Sandbach		GB	

US-CL-CURRENT: 435/173.1; 424/93.7

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 10. Document ID: US 20020143170 A1

L5: Entry 10 of 79 File: PGPB Oct 3, 2002

PGPUB-DOCUMENT-NUMBER: 20020143170
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020143170 A1

TITLE: Bone morphogenic protein (BMP) polynucleotides, polypeptides, and antibodies

PUBLICATION-DATE: October 3, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Ni, Jian	Germantown	MD	US	
Ruben, Steven M.	Olney	MD	US	
Shi, Yanggu	Gaithersburg	MD	US	

US-CL-CURRENT: 536/23.2; 435/226, 435/320.1, 435/325, 435/6, 435/69.1, 435/7.1, 530/388.26

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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Term	Documents
BRIDG$	0
BRIDG.DWPI,EPAB,JPAB,USPT,PGPB.	49
BRIDGA.DWPI,EPAB,JPAB,USPT,PGPB.	1
BRIDGABILITY.DWPI,EPAB,JPAB,USPT,PGPB.	7
BRIDGABLE.DWPI,EPAB,JPAB,USPT,PGPB.	32
BRIDGADE.DWPI,EPAB,JPAB,USPT,PGPB.	4
BRIDGALL.DWPI,EPAB,JPAB,USPT,PGPB.	11
BRIDGALL-ET-AL.DWPI,EPAB,JPAB,USPT,PGPB.	11
BRIDGAM.DWPI,EPAB,JPAB,USPT,PGPB.	1
BRIDGAMAN.DWPI,EPAB,JPAB,USPT,PGPB.	1
BRIDGBON.DWPI,EPAB,JPAB,USPT,PGPB.	1
(L4 AND BRIDG$).USPT,PGPB,JPAB,EPAB,DWPI.	79

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Search Results - Record(s) 11 through 20 of 79 returned.

☐ 11. Document ID: US 20020142461 A1

L5: Entry 11 of 79 File: PGPB Oct 3, 2002

PGPUB-DOCUMENT-NUMBER: 20020142461
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020142461 A1

TITLE: T1 Receptor-like ligand II and uses thereof

PUBLICATION-DATE: October 3, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Ni, Jian	Rockville	MD	US	
Gentz, Reiner L.	Rockville	MD	US	
Ruben, Steven M.	Olney	MD	US	

US-CL-CURRENT: 435/372; 424/85.1, 514/2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 12. Document ID: US 20020115112 A1

L5: Entry 12 of 79 File: PGPB Aug 22, 2002

PGPUB-DOCUMENT-NUMBER: 20020115112
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020115112 A1

TITLE: Neutrokine-alpha and Neutrokine-alpha splice variant

PUBLICATION-DATE: August 22, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Yu, Guo-Liang	Berkeley	CA	US	
Ebner, Reinhard	Gaithersburg	MD	US	
Ni, Jian	Germantown	MD	US	
Rosen, Craig A.	Laytonsville	MD	US	
Ullrich, Stephen	Rockville	MD	US	

US-CL-CURRENT: 435/7.2; 424/145.1, 530/388.23

[redacted]

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 13. Document ID: US 20020111471 A1

L5: Entry 13 of 79 File: PGPB Aug 15, 2002

PGPUB-DOCUMENT-NUMBER: 20020111471
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020111471 A1

TITLE: Compositions and methods for diagnosing and treating conditions, disorders, or diseases involving cell death

PUBLICATION-DATE: August 15, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Lo, Donald C.	Chapel Hill	NC	US	
Barney, Shawn	Apex	NC	US	
Thomas, Mary Beth	Chapel Hill	NC	US	
Portbury, Stuart D.	Durham	NC	US	
Puranam, Kasturi	Durham	NC	US	
Katz, Lawrence C.	Durham	NC	US	

US-CL-CURRENT: 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 14. Document ID: US 20020107211 A1

L5: Entry 14 of 79 File: PGPB Aug 8, 2002

PGPUB-DOCUMENT-NUMBER: 20020107211
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020107211 A1

TITLE: Modulators of body weight, corresponding nucleic acids and proteins, and diagnostic and therapeutic uses thereof

PUBLICATION-DATE: August 8, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Friedman, Jeffrey M.	New York	NY	US	
Halaas, Jeffrey L.	New York	NY	US	
Gajiwala, Ketan	New York	NY	US	
Burley, Stephen K.	New York	NY	US	
Zhang, Yiying	New York	NY	US	
Proenca, Ricardo	Astoria	NY	US	
Maffei, Margherita	New York	NY	US	

US-CL-CURRENT: 514/44; 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☐ 15. Document ID: US 20020106739 A1

L5: Entry 15 of 79 File: PGPB Aug 8, 2002

PGPUB-DOCUMENT-NUMBER: 20020106739
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020106739 A1

TITLE: Modified G-protein coupled receptors

PUBLICATION-DATE: August 8, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Oakley, Robert H.	Durham	NC	US	
Barak, Lawrence S.	Durham	NC	US	
Laporte, Stephane A.	Outremont	NC	CA	
Caron, Marc G.	Hillsborough		US	

US-CL-CURRENT: 435/69.1; 435/320.1, 435/325, 530/350, 536/23.5

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☐ 16. Document ID: US 20020106736 A1

L5: Entry 16 of 79 File: PGPB Aug 8, 2002

PGPUB-DOCUMENT-NUMBER: 20020106736
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020106736 A1

TITLE: Human tumor necrosis factor receptor TR17

PUBLICATION-DATE: August 8, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Ruben, Steven M.	Olney	MD	US	
Baker, Kevin P.	Darnestown	MD	US	

US-CL-CURRENT: 435/69.1; 435/320.1, 435/325, 530/350, 536/23.5

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☐ 17. Document ID: US 20020098473 A1

L5: Entry 17 of 79 File: PGPB Jul 25, 2002

PGPUB-DOCUMENT-NUMBER: 20020098473
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020098473 A1

TITLE: Novel glutamate transporters

PUBLICATION-DATE: July 25, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Edwards, Robert H.	San Francisco	CA	US	
Bellocchio, Elizabeth E.	Walnut Creek	CA	US	
Fremeau, Robert T. JR.	San Francisco	CA	US	
Reimer, Richard J.	San Francisco	CA	US	

US-CL-CURRENT: 435/4; 435/6

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 18. Document ID: US 20020098163 A1

L5: Entry 18 of 79 File: PGPB Jul 25, 2002

PGPUB-DOCUMENT-NUMBER: 20020098163
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020098163 A1

TITLE: Human tumor necrosis factor receptors TR21 and TR22

PUBLICATION-DATE: July 25, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Zeng, Zhi-Zhen	Lansdale	PA	US	
Ruben, Steven M.	Olney	MD	US	
Rosen, Craig A.	Laytonsville	MD	US	

US-CL-CURRENT: 424/85.1; 435/320.1, 435/325, 435/69.1, 530/350, 536/23.5

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 19. Document ID: US 20020076795 A1

L5: Entry 19 of 79 File: PGPB Jun 20, 2002

PGPUB-DOCUMENT-NUMBER: 20020076795
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020076795 A1

TITLE: Novel TRF1 binding protein, methods of use thereof

[redacted]

PUBLICATION-DATE: June 20, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Lange, Titia De	New York	NY	US	
Smith, Susan	New York	NY	US	

US-CL-CURRENT: 435/226; 435/320.1, 435/325, 435/69.1, 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☐ 20. Document ID: US 20020076719 A1

L5: Entry 20 of 79 File: PGPB Jun 20, 2002

PGPUB-DOCUMENT-NUMBER: 20020076719
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020076719 A1

TITLE: Telomere repeat binding factors and diagnostic and therapeutic use thereof

PUBLICATION-DATE: June 20, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Lange, Titia De	New York	NY	US	
Broccoli, Dominique	New York	NY	US	
Smogorzenska, Agata	New York	NY	US	

US-CL-CURRENT: 435/6; 435/199, 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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[redacted]

Term	Documents
BRIDG$	0
BRIDG.DWPI,EPAB,JPAB,USPT,PGPB.	49
BRIDGA.DWPI,EPAB,JPAB,USPT,PGPB.	1
BRIDGABILITY.DWPI,EPAB,JPAB,USPT,PGPB.	7
BRIDGABLE.DWPI,EPAB,JPAB,USPT,PGPB.	32
BRIDGADE.DWPI,EPAB,JPAB,USPT,PGPB.	4
BRIDGALL.DWPI,EPAB,JPAB,USPT,PGPB.	11
BRIDGALL-ET-AL.DWPI,EPAB,JPAB,USPT,PGPB.	11
BRIDGAM.DWPI,EPAB,JPAB,USPT,PGPB.	1
BRIDGAMAN.DWPI,EPAB,JPAB,USPT,PGPB.	1
BRIDGBON.DWPI,EPAB,JPAB,USPT,PGPB.	1
(L4 AND BRIDG$).USPT,PGPB,JPAB,EPAB,DWPI.	79

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Search Results - Record(s) 21 through 30 of 79 returned.

☐ 21. Document ID: US 20020072091 A1

L5: Entry 21 of 79 File: PGPB Jun 13, 2002

PGPUB-DOCUMENT-NUMBER: 20020072091
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020072091 A1

TITLE: Death domain containing receptor 5

PUBLICATION-DATE: June 13, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Ni, Jian	Rockville	MD	US	
Gentz, Reiner L.	Rockville	MD	US	
Yu, Guo-Liang	Berkeley	CA	US	
Rosen, Craig A.	Laytonville	MD	US	

US-CL-CURRENT: 435/69.1; 435/320.1, 435/325, 530/350, 536/23.5

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 22. Document ID: US 20020068319 A1

L5: Entry 22 of 79 File: PGPB Jun 6, 2002

PGPUB-DOCUMENT-NUMBER: 20020068319
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020068319 A1

TITLE: 32 human secreted proteins

PUBLICATION-DATE: June 6, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Ni, Jian	Germantown	MD	US	
Baker, Kevin P.	Darnestown	MD	US	
Birse, Charles E.	North Potomac	MD	US	
Ebner, Reinhard	Gaithersburg	MD	US	
Fiscella, Michele	Bethesda	MD	US	
Komatsoulis, George A.	Silver Spring	MD	US	
LaFleur, David W.	Washington	DC	US	
Moore, Paul A.	Germantown	MD	US	
Olsen, Henrik S.	Gaithersburg	MD	US	
Rosen, Craig A.	Laytonsville	MD	US	
Ruben, Steven M.	Olney	MD	US	
Soppet, Daniel R.	Centreville	VA	US	
Young, Paul E.	Gaithersburg	MD	US	
Wei, Ping	Brookeville	MD	US	
Florence, Kimberly A.	Rockville	MD	US	

US-CL-CURRENT: 435/69.1; 435/183, 435/325, 435/7.1, 530/388.1, 536/23.5

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☐ 23. Document ID: US 20020064829 A1

L5: Entry 23 of 79 File: PGPB May 30, 2002

PGPUB-DOCUMENT-NUMBER: 20020064829
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020064829 A1

TITLE: Human tumor necrosis factor delta and epsilon

PUBLICATION-DATE: May 30, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Yu, Guo-Liang	Berkeley	CA	US	
Ni, Jian	Germantown	MD	US	
Gentz, Reiner L.	Rockville	MD	US	
Dillon, Patrick J.	Carlsbad	CA	US	

US-CL-CURRENT: 435/69.1; 424/145.1, 435/320.1, 435/325, 530/351, 530/388.23, 536/23.5

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☐ 24. Document ID: US 20020064811 A1

L5: Entry 24 of 79 File: PGPB May 30, 2002

PGPUB-DOCUMENT-NUMBER: 20020064811

PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020064811 A1

TITLE: DNA ENCODING THE HUMAN SYNAPSIN III GENE AND USES THEREOF

PUBLICATION-DATE: May 30, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
GREENGARD, PAUL	NEW YORK	NY	US	
PORTON, BARBARA	NEW YORK	NY	US	
KAO, HUNG-TEH	NEW YORK	NY	US	

US-CL-CURRENT: 435/69.1; 435/320.1, 435/325, 435/348, 435/352, 435/358, 435/362, 435/366, 435/368, 435/69.3, 536/23.1, 536/23.5, 536/24.31

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 25. Document ID: US 20020061304 A1

L5: Entry 25 of 79 File: PGPB May 23, 2002

PGPUB-DOCUMENT-NUMBER: 20020061304
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020061304 A1

TITLE: Combined treatment with keratinocyte growth factor and epidermal growth factor inhibitor

PUBLICATION-DATE: May 23, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Miller, Penelope Elizabeth	Mystic	CT	US	
Moyer, James Dale	East Lyme	CT	US	

US-CL-CURRENT: 424/143.1; 514/262.1, 514/264.1, 514/265.1, 514/266.4

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 26. Document ID: US 20020052015 A1

L5: Entry 26 of 79 File: PGPB May 2, 2002

PGPUB-DOCUMENT-NUMBER: 20020052015
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020052015 A1

TITLE: Methuselah gene, compositions and methods of use

PUBLICATION-DATE: May 2, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Lin, Yi-Jyun	Arcadia	CA	US	
Benzer, Seymour	San Marino	CA	US	

US-CL-CURRENT: 435/7.92; 530/388.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 27. Document ID: US 20020028449 A1

L5: Entry 27 of 79 File: PGPB Mar 7, 2002

PGPUB-DOCUMENT-NUMBER: 20020028449
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020028449 A1

TITLE: 26 Human secreted proteins

PUBLICATION-DATE: March 7, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Ruben, Steven M.	Olney	MD	US	
Birse, Charles E.	North Potomac	MD	US	
Duan, Roxanne D.	Bethesda	MD	US	
Soppet, Daniel R.	Centreville	VA	US	
Rosen, Craig A.	Laytonsville	MD	US	
Shi, Yanggu	Gaithersburg	MD	US	
LaFleur, David W.	Washington	DC	US	
Olsen, Henrik	Gaithersburg	MD	US	
Ebner, Reinhard	Gaithersburg	MD	US	
Florence, Kimberly A.	Rockville	MD	US	
Ni, Jian	Rockville	MD	US	
Young, Paul	Gaithersburg	MD	US	

US-CL-CURRENT: 435/6; 435/183, 435/69.1, 530/388.1, 536/23.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 28. Document ID: US 20010053549 A1

L5: Entry 28 of 79 File: PGPB Dec 20, 2001

PGPUB-DOCUMENT-NUMBER: 20010053549
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20010053549 A1

TITLE: Loading method

PUBLICATION-DATE: December 20, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
McHale, Anthony P.	Portstewart		GB	
Craig, Roger	Smallwood		GB	
Fadlon, Emma Jane	Portstewart		GB	

US-CL-CURRENT: 435/446; 435/173.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☐ 29. Document ID: US 20010008758 A1

L5: Entry 29 of 79 File: PGPB Jul 19, 2001

PGPUB-DOCUMENT-NUMBER: 20010008758
PGPUB-FILING-TYPE: new-utility
DOCUMENT-IDENTIFIER: US 20010008758 A1

TITLE: Delivery of an agent

PUBLICATION-DATE: July 19, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
McHale, Anthony Patrick	Portstewart		GB	
Craig, Roger	Sandbach Cheshire		GB	
Haro, Ana Maria Rollan	Londonberry		GB	

US-CL-CURRENT: 435/2; 424/93.7, 435/325

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☐ 30. Document ID: US 6476193 B1

L5: Entry 30 of 79 File: USPT Nov 5, 2002

US-PAT-NO: 6476193
DOCUMENT-IDENTIFIER: US 6476193 B1

TITLE: NLK1 protein and NLK1 protein complexes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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Generate Collection Print

Term	Documents
BRIDG$	0
BRIDG.DWPI,EPAB,JPAB,USPT,PGPB.	49
BRIDGA.DWPI,EPAB,JPAB,USPT,PGPB.	1
BRIDGABILITY.DWPI,EPAB,JPAB,USPT,PGPB.	7
BRIDGABLE.DWPI,EPAB,JPAB,USPT,PGPB.	32
BRIDGADE.DWPI,EPAB,JPAB,USPT,PGPB.	4
BRIDGALL.DWPI,EPAB,JPAB,USPT,PGPB.	11
BRIDGALL-ET-AL.DWPI,EPAB,JPAB,USPT,PGPB.	11
BRIDGAM.DWPI,EPAB,JPAB,USPT,PGPB.	1
BRIDGAMAN.DWPI,EPAB,JPAB,USPT,PGPB.	1
BRIDGBON.DWPI,EPAB,JPAB,USPT,PGPB.	1
(L4 AND BRIDG$).USPT,PGPB,JPAB,EPAB,DWPI.	79

There are more results than shown above. Click here to view the entire set.

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[redacted]

WEST

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Search Results - Record(s) 31 through 40 of 79 returned.

☐ 31. Document ID: US 6471956 B1

L5: Entry 31 of 79 File: USPT Oct 29, 2002

US-PAT-NO: 6471956
DOCUMENT-IDENTIFIER: US 6471956 B1

TITLE: Ob polypeptides, modified forms and compositions thereto

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 32. Document ID: US 6440696 B1

L5: Entry 32 of 79 File: USPT Aug 27, 2002

US-PAT-NO: 6440696
DOCUMENT-IDENTIFIER: US 6440696 B1

TITLE: E6 targeted protein (E6TP1)

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 33. Document ID: US 6429290 B1

L5: Entry 33 of 79 File: USPT Aug 6, 2002

US-PAT-NO: 6429290
DOCUMENT-IDENTIFIER: US 6429290 B1

TITLE: OB polypeptides, modified forms and derivatives

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 34. Document ID: US 6429010 B1

L5: Entry 34 of 79 File: USPT Aug 6, 2002

US-PAT-NO: 6429010
DOCUMENT-IDENTIFIER: US 6429010 B1

TITLE: DNA encoding the human synapsin III gene and uses thereof

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 35. Document ID: US 6406867 B1

L5: Entry 35 of 79 File: USPT Jun 18, 2002

US-PAT-NO: 6406867
DOCUMENT-IDENTIFIER: US 6406867 B1

TITLE: Antibody to human endokine alpha and methods of use

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 36. Document ID: US 6403770 B1

L5: Entry 36 of 79 File: USPT Jun 11, 2002

US-PAT-NO: 6403770
DOCUMENT-IDENTIFIER: US 6403770 B1

TITLE: Antibodies to neutrokine-alpha

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 37. Document ID: US 6350730 B1

L5: Entry 37 of 79 File: USPT Feb 26, 2002

US-PAT-NO: 6350730
DOCUMENT-IDENTIFIER: US 6350730 B1

TITLE: OB polypeptides and modified forms as modulators of body weight

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 38. Document ID: US 6346605 B1

L5: Entry 38 of 79 File: USPT Feb 12, 2002

US-PAT-NO: 6346605
DOCUMENT-IDENTIFIER: US 6346605 B1

TITLE: Signal transducer for the TNF receptor super family, and uses thereof

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

[redacted]

☐ 39. Document ID: US 6338949 B1

L5: Entry 39 of 79 File: USPT Jan 15, 2002

US-PAT-NO: 6338949
DOCUMENT-IDENTIFIER: US 6338949 B1

TITLE: Nucleic acids encoding receptor recognition factor stat4 and methods of use thereof

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 40. Document ID: US 6322976 B1

L5: Entry 40 of 79 File: USPT Nov 27, 2001

US-PAT-NO: 6322976
DOCUMENT-IDENTIFIER: US 6322976 B1

TITLE: Compositions and methods of disease diagnosis and therapy

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

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Term	Documents
BRIDG$	0
BRIDG.DWPI,EPAB,JPAB,USPT,PGPB.	49
BRIDGA.DWPI,EPAB,JPAB,USPT,PGPB.	1
BRIDGABILITY.DWPI,EPAB,JPAB,USPT,PGPB.	7
BRIDGABLE.DWPI,EPAB,JPAB,USPT,PGPB.	32
BRIDGADE.DWPI,EPAB,JPAB,USPT,PGPB.	4
BRIDGALL.DWPI,EPAB,JPAB,USPT,PGPB.	11
BRIDGALL-ET-AL.DWPI,EPAB,JPAB,USPT,PGPB.	11
BRIDGAM.DWPI,EPAB,JPAB,USPT,PGPB.	1
BRIDGAMAN.DWPI,EPAB,JPAB,USPT,PGPB.	1
BRIDGBON.DWPI,EPAB,JPAB,USPT,PGPB.	1
(L4 AND BRIDG$).USPT,PGPB,JPAB,EPAB,DWPI.	79

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Search Results - Record(s) 41 through 50 of 79 returned.

☐ 41. Document ID: US 6316596 B1

L5: Entry 41 of 79 File: USPT Nov 13, 2001

US-PAT-NO: 6316596
DOCUMENT-IDENTIFIER: US 6316596 B1

TITLE: Receptor for peptide hormones involving in energy homeostasis

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 42. Document ID: US 6316222 B1

L5: Entry 42 of 79 File: USPT Nov 13, 2001

US-PAT-NO: 6316222
DOCUMENT-IDENTIFIER: US 6316222 B1

TITLE: Nucleic acids encoding a lymphocyte surface receptor that binds CAML

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 43. Document ID: US 6309853 B1

L5: Entry 43 of 79 File: USPT Oct 30, 2001

US-PAT-NO: 6309853
DOCUMENT-IDENTIFIER: US 6309853 B1

TITLE: Modulators of body weight, corresponding nucleic acids and proteins, and diagnostic and therapeutic uses thereof

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 44. Document ID: US 6307025 B1

L5: Entry 44 of 79 File: USPT Oct 23, 2001

US-PAT-NO: 6307025
DOCUMENT-IDENTIFIER: US 6307025 B1

TITLE: VCAM fusion proteins and DNA coding therefor

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 45. Document ID: US 6303768 B1

L5: Entry 45 of 79 File: USPT Oct 16, 2001

US-PAT-NO: 6303768
DOCUMENT-IDENTIFIER: US 6303768 B1

TITLE: Methuselah gene, compositions and methods of use

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 46. Document ID: US 6297356 B1

L5: Entry 46 of 79 File: USPT Oct 2, 2001

US-PAT-NO: 6297356
DOCUMENT-IDENTIFIER: US 6297356 B1

TITLE: Telomere repeat binding factors and diagnostic and therapeutic use thereof

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 47. Document ID: US 6277974 B1

L5: Entry 47 of 79 File: USPT Aug 21, 2001

US-PAT-NO: 6277974
DOCUMENT-IDENTIFIER: US 6277974 B1

TITLE: Compositions and methods for diagnosing and treating conditions, disorders, or diseases involving cell death

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 48. Document ID: US 6277613 B1

L5: Entry 48 of 79 File: USPT Aug 21, 2001

US-PAT-NO: 6277613
DOCUMENT-IDENTIFIER: US 6277613 B1

TITLE: TRF1 binding protein, methods of use thereof

[redacted]

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 49. Document ID: US 6252043 B1

L5: Entry 49 of 79 File: USPT Jun 26, 2001

US-PAT-NO: 6252043
DOCUMENT-IDENTIFIER: US 6252043 B1

TITLE: Vascular cell adhesion molecule (VCAM) polypeptides

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 50. Document ID: US 6225063 B1

L5: Entry 50 of 79 File: USPT May 1, 2001

US-PAT-NO: 6225063
DOCUMENT-IDENTIFIER: US 6225063 B1

TITLE: RNA channels in biological membranes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

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Term	Documents
BRIDG$	0
BRIDG.DWPI,EPAB,JPAB,USPT,PGPB.	49
BRIDGA.DWPI,EPAB,JPAB,USPT,PGPB.	1
BRIDGABILITY.DWPI,EPAB,JPAB,USPT,PGPB.	7
BRIDGABLE.DWPI,EPAB,JPAB,USPT,PGPB.	32
BRIDGADE.DWPI,EPAB,JPAB,USPT,PGPB.	4
BRIDGALL.DWPI,EPAB,JPAB,USPT,PGPB.	11
BRIDGALL-ET-AL.DWPI,EPAB,JPAB,USPT,PGPB.	11
BRIDGAM.DWPI,EPAB,JPAB,USPT,PGPB.	1
BRIDGAMAN.DWPI,EPAB,JPAB,USPT,PGPB.	1
BRIDGBON.DWPI,EPAB,JPAB,USPT,PGPB.	1
(L4 AND BRIDG$).USPT,PGPB,JPAB,EPAB,DWPI.	79

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Search Results - Record(s) 51 through 60 of 79 returned.

☐ 51. Document ID: US 6177259 B1

L5: Entry 51 of 79 File: USPT Jan 23, 2001

US-PAT-NO: 6177259
DOCUMENT-IDENTIFIER: US 6177259 B1

TITLE: Assays and kits for inhibition of polyglutamine-induced cell death

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 52. Document ID: US 6162796 A

L5: Entry 52 of 79 File: USPT Dec 19, 2000

US-PAT-NO: 6162796
DOCUMENT-IDENTIFIER: US 6162796 A

TITLE: Method for transferring genes to the heart using AAV vectors

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 53. Document ID: US 6124448 A

L5: Entry 53 of 79 File: USPT Sep 26, 2000

US-PAT-NO: 6124448
DOCUMENT-IDENTIFIER: US 6124448 A

TITLE: Nucleic acid primers and probes for the mammalian OB gene

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 54. Document ID: US 6124439 A

L5: Entry 54 of 79 File: USPT Sep 26, 2000

US-PAT-NO: 6124439
DOCUMENT-IDENTIFIER: US 6124439 A

TITLE: OB polypeptide antibodies and method of making

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 55. Document ID: US 6124118 A

L5: Entry 55 of 79 File: USPT Sep 26, 2000

US-PAT-NO: 6124118
DOCUMENT-IDENTIFIER: US 6124118 A

TITLE: Receptor recognition factors, protein sequences and methods of use thereof

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 56. Document ID: US 6123923 A

L5: Entry 56 of 79 File: USPT Sep 26, 2000

US-PAT-NO: 6123923
DOCUMENT-IDENTIFIER: US 6123923 A

TITLE: Optoacoustic contrast agents and methods for their use

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 57. Document ID: US 6060590 A

L5: Entry 57 of 79 File: USPT May 9, 2000

US-PAT-NO: 6060590
DOCUMENT-IDENTIFIER: US 6060590 A

TITLE: Chitinase related proteins and methods of use

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 58. Document ID: US 6048837 A

L5: Entry 58 of 79 File: USPT Apr 11, 2000

US-PAT-NO: 6048837
DOCUMENT-IDENTIFIER: US 6048837 A

TITLE: OB polypeptides as modulators of body weight

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 59. Document ID: US 6040168 A

L5: Entry 59 of 79 File: USPT Mar 21, 2000

US-PAT-NO: 6040168
DOCUMENT-IDENTIFIER: US 6040168 A

TITLE: DNA encoding the human synapsin III gene and uses thereof

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 60. Document ID: US 6030808 A

L5: Entry 60 of 79 File: USPT Feb 29, 2000

US-PAT-NO: 6030808
DOCUMENT-IDENTIFIER: US 6030808 A

TITLE: Nucleic acids encoding receptor recognition factor stat 3 and methods of use thereof

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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Term	Documents
BRIDG$	0
BRIDG.DWPI,EPAB,JPAB,USPT,PGPB.	49
BRIDGA.DWPI,EPAB,JPAB,USPT,PGPB.	1
BRIDGABILITY.DWPI,EPAB,JPAB,USPT,PGPB.	7
BRIDGABLE.DWPI,EPAB,JPAB,USPT,PGPB.	32
BRIDGADE.DWPI,EPAB,JPAB,USPT,PGPB.	4
BRIDGALL.DWPI,EPAB,JPAB,USPT,PGPB.	11
BRIDGALL-ET-AL.DWPI,EPAB,JPAB,USPT,PGPB.	11
BRIDGAM.DWPI,EPAB,JPAB,USPT,PGPB.	1
BRIDGAMAN.DWPI,EPAB,JPAB,USPT,PGPB.	1
BRIDGBON.DWPI,EPAB,JPAB,USPT,PGPB.	1
(L4 AND BRIDG$).USPT,PGPB,JPAB,EPAB,DWPI.	79

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Search Results - Record(s) 61 through 70 of 79 returned.

☐ 61. Document ID: US 6013515 A

L5: Entry 61 of 79 File: USPT Jan 11, 2000

US-PAT-NO: 6013515
DOCUMENT-IDENTIFIER: US 6013515 A

TITLE: Cofactors for HIV-1 protein Tat and methods of use therefor

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 62. Document ID: US 6013475 A

L5: Entry 62 of 79 File: USPT Jan 11, 2000

US-PAT-NO: 6013475
DOCUMENT-IDENTIFIER: US 6013475 A

TITLE: Nucleic acids encoding receptor recognition factors and methods of use thereof

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 63. Document ID: US 6001968 A

L5: Entry 63 of 79 File: USPT Dec 14, 1999

US-PAT-NO: 6001968
DOCUMENT-IDENTIFIER: US 6001968 A

TITLE: OB polypeptides, modified forms and compositions

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 64. Document ID: US 5986055 A

L5: Entry 64 of 79 File: USPT Nov 16, 1999

US-PAT-NO: 5986055
DOCUMENT-IDENTIFIER: US 5986055 A

TITLE: CDK2 interactions

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 65. Document ID: US 5977311 A

L5: Entry 65 of 79 File: USPT Nov 2, 1999

US-PAT-NO: 5977311
DOCUMENT-IDENTIFIER: US 5977311 A

TITLE: 53BP2 complexes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 66. Document ID: US 5976835 A

L5: Entry 66 of 79 File: USPT Nov 2, 1999

US-PAT-NO: 5976835
DOCUMENT-IDENTIFIER: US 5976835 A

TITLE: Nucleic acids encoding receptor recognition factor Stat1.alpha. and Stat1.beta., and methods of use thereof

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 67. Document ID: US 5969102 A

L5: Entry 67 of 79 File: USPT Oct 19, 1999

US-PAT-NO: 5969102
DOCUMENT-IDENTIFIER: US 5969102 A

TITLE: Lymphocyte surface receptor that binds CAML, nucleic acids encoding the same and methods of use thereof

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 68. Document ID: US 5955579 A

L5: Entry 68 of 79 File: USPT Sep 21, 1999

US-PAT-NO: 5955579
DOCUMENT-IDENTIFIER: US 5955579 A

TITLE: Islet-specific homeoprotein and transcriptional regulator of insulin gene expression, HOXB13

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 69. Document ID: US 5935810 A

L5: Entry 69 of 79 File: USPT Aug 10, 1999

US-PAT-NO: 5935810
DOCUMENT-IDENTIFIER: US 5935810 A

TITLE: Mammalian ob polypeptides capable of modulating body weight, corresponding nucleic acids, and diagnostic and therapeutic uses thereof

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 70. Document ID: US 5891720 A

L5: Entry 70 of 79 File: USPT Apr 6, 1999

US-PAT-NO: 5891720
DOCUMENT-IDENTIFIER: US 5891720 A

TITLE: Isolated DNA encoding a novel human G-protein coupled receptor

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

Generate Collection | Print

Term	Documents
BRIDG$	0
BRIDG.DWPI,EPAB,JPAB,USPT,PGPB.	49
BRIDGA.DWPI,EPAB,JPAB,USPT,PGPB.	1
BRIDGABILITY.DWPI,EPAB,JPAB,USPT,PGPB.	7
BRIDGABLE.DWPI,EPAB,JPAB,USPT,PGPB.	32
BRIDGADE.DWPI,EPAB,JPAB,USPT,PGPB.	4
BRIDGALL.DWPI,EPAB,JPAB,USPT,PGPB.	11
BRIDGALL-ET-AL.DWPI,EPAB,JPAB,USPT,PGPB.	11
BRIDGAM.DWPI,EPAB,JPAB,USPT,PGPB.	1
BRIDGAMAN.DWPI,EPAB,JPAB,USPT,PGPB.	1
BRIDGBON.DWPI,EPAB,JPAB,USPT,PGPB.	1
(L4 AND BRIDG$).USPT,PGPB,JPAB,EPAB,DWPI.	79

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☐ 71. Document ID: US 5885831 A

L5: Entry 71 of 79 File: USPT Mar 23, 1999

US-PAT-NO: 5885831
DOCUMENT-IDENTIFIER: US 5885831 A

TITLE: Nuclear localization factor associated with circadian rhythms

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 72. Document ID: US 5882899 A

L5: Entry 72 of 79 File: USPT Mar 16, 1999

US-PAT-NO: 5882899
DOCUMENT-IDENTIFIER: US 5882899 A

TITLE: Receptor for peptide hormones involved in energy homeostasis, and method and compositions for use thereof

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 73. Document ID: US 5846800 A

L5: Entry 73 of 79 File: USPT Dec 8, 1998

US-PAT-NO: 5846800
DOCUMENT-IDENTIFIER: US 5846800 A

TITLE: Nucleic acid molecules encoding a novel receptor-type protein tyrosine phosphatase-.sigma.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 74. Document ID: US 5840842 A

L5: Entry 74 of 79 File: USPT Nov 24, 1998

US-PAT-NO: 5840842
DOCUMENT-IDENTIFIER: US 5840842 A

[redacted]

TITLE: Receptor-type phosphotyrosine phosphatase-sigma

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 75. Document ID: US 5831051 A

L5: Entry 75 of 79 File: USPT Nov 3, 1998

US-PAT-NO: 5831051
DOCUMENT-IDENTIFIER: US 5831051 A

TITLE: Receptor for peptide hormones involved in energy homeostasis, and method and compositions for use thereof

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 76. Document ID: US 5733730 A

L5: Entry 76 of 79 File: USPT Mar 31, 1998

US-PAT-NO: 5733730
DOCUMENT-IDENTIFIER: US 5733730 A

TITLE: Telomere repeat binding factor and diagnostic and therapeutic use thereof

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 77. Document ID: US 5367056 A

L5: Entry 77 of 79 File: USPT Nov 22, 1994

US-PAT-NO: 5367056
DOCUMENT-IDENTIFIER: US 5367056 A

TITLE: Endothelial cell-leukocyte adhesion molecules (ELAMs) and molecules involved in leukocyte adhesion (MILAs)

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 78. Document ID: US 5272263 A

L5: Entry 78 of 79 File: USPT Dec 21, 1993

US-PAT-NO: 5272263
DOCUMENT-IDENTIFIER: US 5272263 A

TITLE: DNA sequences encoding vascular cell adhesion molecules (VCAMS)

[redacted]

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 79. Document ID: JP 2002528109 W WO 200026226 A1 AU 200016013 A EP 1133513 A1

L5: Entry 79 of 79 File: DWPI Sep 3, 2002

DERWENT-ACC-NO: 2000-365558
DERWENT-WEEK: 200273

TITLE: Novel functional polynucleotides comprising an actuator domain, a receptor domain, and a bridging domain useful for generating highly specific polynucleotide sensors and as genetic control elements

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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(L4 AND BRIDG$).USPT,PGPB,JPAB,EPAB,DWPI.	79

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L5: Entry 50 of 79 File: USPT May 1, 2001

DOCUMENT-IDENTIFIER: US 6225063 B1
TITLE: RNA channels in biological membranes

Brief Summary Text (15):
In addition, the nucleic acid molecules of the present invention are not limited to any particular length. In some embodiments of the present invention, the nucleic acid molecules are from about 10 to 2500 nucleotides in length. In other embodiments, the nucleic acid molecules are from about 20 to 500 nucleotides in length. In still other embodiments, the present invention provides nucleic acid molecules from about 30 to 150 nucleotides in length. In further embodiments, the nucleic acid molecules are from about 40 to 120 nucleotides in length. In some preferred embodiments, the nucleic acids are from about 50 to 100 nucleotides in length. In a particularly preferred embodiment, the nucleic acid molecules are about 70 to 95 nucleotides in length, while in other particularly preferred embodiments, the nucleic acid molecules are about 50 to 60 nucleotides in length. It will be recognized that the nucleic acids of the present invention may also be included as a domain or portion of a larger nucleic acid molecule.

Brief Summary Text (20):
In further embodiments, the present invention provides compositions comprising nucleic acid molecules capable of binding membranes, wherein the nucleic acid molecules are conjugated or linked to at least one effector molecule or domain. It is not intended that the present invention be limited by the nature of the effector molecule. A variety of effector molecules or domains are contemplated including organic molecules (e.g., biotin), RNA molecules, DNA molecules, toxins (e.g., ricin or restrictocin), growth factors, cytokines, kinases, hormones, antibodies, and other proteins with a desired biological activity. In other embodiments, the effector molecule or domain targets the nucleic acid to a specific cell or organelle type. A variety of such targeting effector domains are contemplated, including, but not limited, to ligands for cell surface receptors, antigen binding proteins directed to cell surface proteins, and RNA and DNA oligonucleotides or polynucleotides capable of binding to cell surface proteins. It is contemplated that utilization of a targeting domain will facilitate targeting of particular cells of interest (e.g., hepatic cells, tumor cells, neurons, etc.).

Brief Summary Text (21):
In some embodiments, the present invention also provides methods for targeting effector molecules to membranes, comprising: providing a membrane preparation, and a composition comprising nucleic acid molecules, wherein the nucleic acid molecules are capable of binding to the membrane, and the nucleic acid molecules are linked to an effector domain; and combining the membrane preparation and nucleic acid molecule such that the nucleic acid molecules bind to membrane preparations.

Detailed Description Text (81):
Accordingly, the present invention contemplates screening a randomized pool of nucleic acid molecules for the ability to bind to membranes. In some embodiments, a composition comprising nucleic acids is provided. In some embodiments, the mixture comprises greater than about 10.sup.12 different nucleic acid sequences, while in particularly preferred embodiments, the mixture comprises greater than about 10.sup.18 different nucleic acid sequences. In preferred embodiments of the present invention, the nucleic acids include a randomized portion. In other embodiments, the randomized portion is from about 30 to 150 nucleotides in length. In still other

embodiments, the randomized portion is from about 40 to 120 nucleotides in length. In other preferred embodiments, the randomized portion is from about 50 to 100 nucleotides in length. In some particularly preferred embodiments, the randomized portion is from about 70 to 95 nucleotides in length, while in other particularly preferred embodiments, the randomized portion is from about 50 to 60 nucleotides in length. However, it is not intended that the nucleic acids of the present invention be limited to the sequences identified by this method. It will be recognized that the nucleic acids of the present invention, and especially the randomized proteins of the nucleic acids of the present invention, may also be included as a domain or portion of a larger nucleic acid molecule.

Detailed Description Text (94):
C. Conjugation of Membrane Binding Domains to other Molecules

Detailed Description Text (95):
In order for many drugs and imaging agents to have therapeutic or diagnostic potential, it is necessary for them to be delivered to the proper location in the body or even more specifically, a cell. The present invention provides compositions and methods for targeting effector molecules to membranes (e.g., biological membranes, such as cellular and organelle membranes) by conjugating the nucleic acid molecules of present invention with effector molecules or domains. Various targeting strategies are known in the art, but these techniques do not employ nucleic acids to bind the cellular membranes. One approach is exemplified by U.S. Pat. No. 4,093,607, herein incorporated by reference, which describes antibodies conjugated to therapeutic agents and methods of employing these constructs in targeting specific cell or tissue antigens. Antibodies conjugated to therapeutic drugs are used to alter the distribution of the therapeutic agent so that it is concentrated at the target cells. Another approach to drug targeting is exemplified by U.S. Pat. No. 5,149,794, herein incorporated by reference, which describes the covalent binding of drugs to a lipid carrier. It is believed that this technique has the advantage of facilitating the entry of drugs that have been specifically targeted to certain cells.

Detailed Description Text (96):
The present invention provides nucleic acid molecules (capable of binding to specific types of cell or tissue membranes) that are conjugated with certain effector (e.g., drug) molecules. It is contemplated that the efficacy of certain drugs can be increased (or lower doses are needed to effect the same result) and that the availability of therapeutic agents to particular cells or tissue types can be increased. Accordingly, in some embodiments, the present invention provides conjugate molecules comprising a nucleic acid membrane binding domain and an effector domain. The effector domain may be a nucleic acid operably linked to the nucleic acid binding domain, or a protein or organic molecule covalently linked to the nucleic acid membrane binding domain.

Detailed Description Text (97):
In further embodiments of the present invention, the effector domain has a catalytic function. In some embodiments, the effector domain comprises a protein having kinase or phosphorylase activity. In other embodiments, the protein has an antioxidant activity (e.g., superoxide dismutase). In still further embodiments, the effector domain comprises an RNA with catalytic activity (i.e., a ribozyme). Ribozymes act by recognizing and then binding a target RNA through complementary base-pairing, and once bound to the correct site, act enzymatically to cut the target RNA. Strategic cleavage of such a target RNA will destroy its ability to direct synthesis of an encoded protein. Examples of ribozymes motifs with enzymatic activity include hammerheads and hairpins (See e.g., U.S. Pat. Nos. 5,891,684; 5,877,022; 5,869,253; 5,811,300; 5,795,778; 5,728,818; and 5,714,383, all of which are incorporated herein by reference).

Detailed Description Text (98):
In still other embodiments of the present invention, the effector domain has a targeting function. Accordingly, in some embodiments, the effector domain comprises an antigen binding protein that recognizes a cell surface or organelle specific antigen. In other embodiments, the effector domain comprises a ligand for a cell surface receptor. In still further embodiments, the effector domain comprises a

signal peptide for a particular organelle. In still other embodiments, the effector domain comprises a ligand binding nucleic acid. Nucleic acids that bind proteins such as reverse transcriptase, Rev and Tat proteins of HIV (Tuerk et al., Gene 137:33 [1993]); human nerve growth factor (Binkley et al., Nucl. Acids Res., 23:3198-205 [1995]); and vascular endothelial growth factor (Jellinek et al., Biochem., 83:10450-6 [1994]), have been identified. In some embodiments of the present invention, the membrane binding nucleic acid is linked to both a catalytic effector domain and targeting effector domain.

Detailed Description Text (99):
When the nucleic acid membrane binding domain and effector domain are both of the same nucleic acid type (e.g., both are RNA or both are DNA), the domains may be operably linked so that only a single nucleic acid molecule need be synthesized. DNA molecules containing both an effector domain and nucleic acid membrane binding domain can be synthesized by PCR or excised from a plasmid by restriction digestion. RNA molecules containing both an effector domain and nucleic acid membrane binding domain can be produced by transcription of a template that encodes both domains (Burke et al., RNA 4:1165 [1998], incorporated herein by reference).

Detailed Description Text (100):
Protein effector domains can be conjugated to nucleic acids (See e.g. Lohse et al., Nature 381:442 [1996]; Zhang et al., Nature 390:96 [1997]; Roberts et al., PNAS 94:12297 [1997]). In some embodiments, a thiol or primary amino group is introduced on the 5' end of an RNA during transcription by an RNA polymerase (e.g., T7 RNA polymerase). In other embodiments, the nucleic acid can be reacted with N-bromoacetyl-N'-phenylalanyl-cystamine to convert the nucleic acid to a 5'-Phe-nucleic acid with a free amino group capable of functioning as an aminoacyl acceptor. Peptides and proteins can be conveniently attached to the thiol or amino group. In other embodiments, the 3' end of a nucleic acid may be modified to contain puromycin, which serves as an peptidyl receptor.

Detailed Description Text (101):
When the effector and nucleic acid membrane-binding domains are of different nucleic acid types (e.g., one is DNA and the other is RNA), the two may be joined together using a bridging oligonucleotide and T4 DNA or RNA ligase (See e.g., Moore et al., Science 256:992 [1992]). In this method, the bridging oligonucleotide is preferably about 20 base pairs in length, and shares a region of complementarity with each of the nucleic acids to be joined. The oligonucleotide hybridizes to each of the nucleic acids to align the ends so that the ligase can join them. This method may also be used to join two RNA molecules.

Detailed Description Text (109):
It is also contemplated that nucleic acids of the present invention will find use in targeting the effector molecules described above to biological membranes. In some embodiments, the membrane binding nucleic acid serves as an "anchor" that is inserted into the cell membrane or membranes of organelles in eukaryotic cells (e.g., membranes of the mitochondria, endoplasmic reticulum, lysomes, and/or nuclei). In some embodiments, the membrane binding nucleic acid serves as an anchor, while a first effector domain linked to the nucleic acid provides cell or target specificity (e.g., an RNA binding motif, an antigen binding protein, or a receptor ligand) and a second effector domain linked to the nucleic acid provides a biological effect (e.g., a ribozyme).

L10: Entry 10 of 14 File: USPT Mar 14, 2000

DOCUMENT-IDENTIFIER: US 6037137 A
TITLE: Fluorogenic peptides for the detection of protease àctivity

Brief Summary Text (23):
This invention also provides a method of detecting a change in conformation of a molecule. The method involves: 1) providing a first molecule having attached thereto a first fluorophore and a second fluorophore wherein the first fluorphore and second fluorophre are the same species of fluorophore, and the fluorophores are juxtaposed at a distance sufficient for the interaction of said fluorophores to detectably reduce the fluorescence intensity of each of said flurophores as compared to the fluoresence intensity of a single fluorophore attached to the molecule at the same location; and 2) detecting the change in fluorescence as the spacing between said fluorophores is increased by a change in conformation of the molecule. Alternatively the change in fluorescence as the relative orientation of the fluorophore is altered by a change in conformation of the molecule may be detected. Preferred fluorophores are capable of forming H-type dimers before the change in conformation. In a preferred embodiment the fluorophores are situated at a distance of less than about 10 angstroms from each other prior to the change in conformation. Particularly preferred fluorophores include the fluorophores described herein. In one embodiment, the change in conformation is cleavage of the molecule into two different molecules each bearing one of the fluorophores. In another embodiment the change in conformation is caused by binding of a target molecule to said first molecule. In one embodiment, the first molecule is a nucleic acid and the change in conformation is produced by hybridization of the nucleic acid to a second nucleic acid or by binding of the nucleic acid to a transcription factor. In another embodiment, the first molecule is a polysaccharide and the change in conformatio nis produced by bindign of an oligosaccharide bindng molecule, e.g., a lecithin binding protein. Preferred "backbone" molecules for this method include a nucleic acid, a polysaccharide, a peptide, a lipid, a protein, a phospholipid, a glycolipid, a glycoprotein, a steroid, or a polymer containign a pH or thiol-sensitive bond where the fluorophores attachment sites sadwich this bond. Where the backbone molecule is a nucleic acid, the change in conformation can be produced by hybridization of the nucleic acid to another nucleic acid or by cleavage of the nucleic acid (e.g., by a restriction endonuclease or a ribozyme). Also the change in conformation can be produced by formation of a complex between the labeled oligonucleotide and a nucleotide binding protein. Where the backbone molecule is a peptide, polysaccharide or a lipid, the change in conformation can be produced by cleavage of the backbone molecule or by complex formation between the backbone molecule and its bindign molecule such as an antibody, receptor, sugar bindign protein, or lipid binding protein.

L10: Entry 9 of 14 File: USPT Jun 27, 2000

DOCUMENT-IDENTIFIER: US 6080851 A
TITLE: Ribozymes with linked anchor sequences

Detailed Description Text (116):
None of the anchor sequences prevented the correct cleavage of the L6 substrate. Kinetic analysis, however, indicated that product release, the rate limiting step in the cleavage reactions, was slower in reactions catalyzed by ribozymes with longer anchors. The initial burst rate was similar for both the 11 nt anchored and the 21 nt anchored ribozyme when reactions were carried out under conditions of substrate excess. These rates are slower than the rates measured for other ribozymes (Proc. Natl. Acad. Sci. USA 1990, 87, 1668-1672; Biochemistry, 1992, 31, 12042-12054.) However, the rates of the anchored ribozymes were measured in reactions using a large substrate RNA molecule while the rates reported for the other ribozymes were determined in reactions using a small oligoribonucleotide substrate. The 21 nt anchored ribozyme was more active than the 11 nt anchored ribozyme when reactions were carried out under conditions of ribozyme excess. Under these conditions, the rate of cleavage by each ribozyme was slower than the rate measured in reactions where substrate was in excess indicating that the ribozymes may undergo a substrate induced conformational change (ie. see Nucleic Acids Res. 1990, 18, 1103-1108. However, for therapeutic purposes, it is advantageous that the ribozymes are more efficient at substrate excess. The 21 nt anchored ribozyme has a slower product dissociation rate than does the 11 nt anchored ribozyme, however, and the net result is that the 11 nt anchored ribozyme is more efficient over an extended reaction time.

L10: Entry 8 of 14 File: USPT Jul 4, 2000

DOCUMENT-IDENTIFIER: US 6083744 A
TITLE: DNA-armed ribozymes and minizymes

Detailed Description Text (101):
The cleavage of the DNA substrate, S21D, by the two ribozymes was performed under conditions where the ribozyme-substrate complex was expected to be fully formed prior to addition of Mg.sup.2+ to initiate the reaction. The substrate in this case was chosen to be 21 nucleotides to compensate for the expected weaker binding of ribozymes to a DNA substrate compared with an RNA substrate. Use of the longer substrate should not result in serious discrepancies in comparing data, since the rate of cleavage of the complex is not expected to depend strongly on the length of the substrate. FIG. 6 shows an example of the data used to determine the rate constants. The data fit the first-order model well. The observed rate constants for cleavage of the DNA substrate are given in the lower half of Table 3. k.sub.2 for ribozyme 2 (0.12 min.sup.-1) is 3 times greater than for ribozyme 1, the same ratio as observed for cleavage of the all-RNA substrate by these ribozymes. The absolute values for rates of cleavage of the DNA substrate are approximately 40-fold less than observed of the DNA substrate are approximately 40-fold less than observed in earlier studies (Yang, et al., 1990; Dahm and Uhlenbeck, 1990). Why should DNA in the substrate decrease the rate of cleavage, but DNA in the arms of the ribozyme increase the rate? It is known that the 2'-hydroxyl of the uridine immediately 5' to the cleavage site on the substrate is involved in binding the Mg.sup.2+ on (Yang, et al., 1990). However, even if both that important uracil and the cytosine at the cleavage site are ribonucleotides, and the remainder are deoxyribonucleotides, such a substrate is still not cleaved as efficiently as an all-RNA substrate (Yang, et al., 1990). Therefore, either there are other 2'-hydroxyl groups in the substrate that are directly involved in stabilizing the active complex, or the structure of the complex formed by the ribozyme and the predominantly DNA substrate is sufficiently different from that formed by the ribozyme with the all-RNA substrate to result in a slightly different arrangement of the crucial groups involved in the reaction. Our observation that activity does not decrease when DNA is substituted into the arms of the ribozyme indicates there are no crucial 2'-hydroxyl groups in the arms, at least in positions 1-10 and 35-42 (FIG. 5). This results is consistent with the observations of Paolella et al. (Paolella, et al., 1992) which showed complete retention of activity by a ribozyme in which all 2'-hydroxyl groups in the hybridizing arms had been replaced by 2'-O-allyl. The fact that the rate of cleavage increases with DNA in the arms of the ribozyme suggest that most likely there is a subtle change in the conformation of the resulting double-helix allowing a more favorable positioning of the critical groups involved in the cleavage reaction. Thus, it may be expected that differences between the effects of all-RNA ribozymes and analogous DNA-armed ribozymes against specific targets will vary with the sequence of the target, as the local structure and flexibility of the helices formed will depend also on the sequence.

L9: Entry 32 of 36 File: USPT Jun 9, 1998

DOCUMENT-IDENTIFIER: US 5763171 A
TITLE: Nucleic acid structures with catalytic and autocatalytic replicating features and methods of use

Brief Summary Text (5):
The term "target" or "target molecule" in a diagnostic sense, refers to a molecule of interest, i.e. the molecule whose presence one wishes to know. In a therapeutic sense, the term "target" or "target molecule" refers to a molecule associated with a disease.

Brief Summary Text (6):
The term "biological binding pair" as used in the present application refers to any pair of molecules which exhibit mutual affinity or binding capacity. A biological binding pair is capable of forming a complex under binding conditions. For the purposes of the present application, the term "ligand" will refer to one molecule of the biological binding pair, and the term "antiligand" or "receptor" will refer to the opposite molecule of the biological binding pair. For example, without limitation, embodiments of the present invention have application in nucleic acid hybridization assays where the biological binding pair includes two complementary nucleic acids. One of the nucleic acids is designated the ligand and the other nucleic acid is designated the antiligand or receptor. One of the nucleic acids may also be a target molecule. The designation of ligand or antiligand is a matter of arbitrary convenience. The biological binding pair may include antigens and antibodies, drugs and drug receptor sites, and enzymes and enzyme substrates, to name a few.

Brief Summary Text (7):
The term "probe" refers to a ligand of known qualities capable of selectively binding to a target antiligand or receptor. As applied to nucleic acids, the term "probe" refers to nucleic acid having a base sequence complementary to a target nucleic acid. The probe and the target are capable of forming a probe target complex under binding conditions. The term "probe" will be used herein, in both a diagnostic sense, meaning capable of binding a molecule, the presence or absence of which one desires to know, and a therapeutic sense, capable of binding to a molecule associated with a disease.

Brief Summary Text (10):
The term "amplify" is used in the broad sense to mean creating an amplification product, which may include by way of example, additional target molecules, or target-like molecules, capable of functioning in a manner like the target molecule, or a molecule subject to detection steps in place of the target molecule, which molecules are created by virtue of the presence of the target molecule in the sample. In the situation where the target is a polynucleotide, additional target, or target-like molecules, or molecules subject to detection can be made enzymatically with DNA or RNA polymerases.

Brief Summary Text (12):
The term "contiguous" means an adjacent area of a molecule. By way of example, in the case of biological binding pairs, where a first ligand binds to a receptor target molecule, the area surrounding and adjacent to the first ligand is open and capable of binding to a second ligand contiguous to the first. In the context of nucleic acid, where a first probe binds to an area of a larger nucleic acid target

molecule, an adjacent mutually exclusive area along the length of the target molecule can bind to a second probe which will then be contiguous to the first. The target molecule acts as a template, directing the position of the first probe and the second probe. The term "substantially contiguous" is used in the functional sense to include spatial orientations which may not touch, may not abut, or may overlap, yet function to bring parts, areas, segments and the like into cooperating relationship.

Brief Summary Text (14):
The term "capture ligand" means a ligand capable of specifically binding with a capture antiligand associated with a support.

Brief Summary Text (17):
The term "reversible," in regard to the binding of ligands and antiligands, means capable of binding or releasing upon imposing changes which do not permanently alter the gross chemical nature of the ligand and antiligand. For example, without limitation, reversible binding would include such binding and release controlled by changes in pH, temperature, and ionic strength which do not destroy the ligand or antiligand.

Brief Summary Text (19):
Molecules of DNA consist of covalently linked chains of deoxyribonucleotides and molecules of RNA consist of covalently linked chains of ribonucleotides. Each nucleic acid is linked by a phosphodiester bridge between the 5'-hydroxyl group of the sugar of one nucleotide and the 3'-hydroxyl group of the sugar of an adjacent nucleotide. The terminal ends of nucleic acid are often referred to as being 5'-termini or 3'-termini in reference to the terminal functional group. Complementary strands of DNA and RNA form antiparallel complexes in which the 3'-terminal end of one strand is oriented and bound to the 5'-terminal end of the opposing strand.

Brief Summary Text (20):
Nucleic acid hybridization assays are based on the tendency of two nucleic acid strands to pair at their complementary regions to form hybrids. The formation of such hybrids can be made to be highly specific by adjustment of the conditions (sometimes referred to as stringency) under which this hybridization takes place such that hybridization will not occur unless the sequences are precisely complementary. If total nucleic acid from the sample is immobilized on a solid support such as a nitrocellulose membrane, the presence of a specific "target" sequence in the sample can be determined by the binding of a complementary nucleic acid "probe" which bears a label. After removal of non-hybridized probe by washing the support, the amount of target is determined by the amount of detectable moiety present.

Brief Summary Text (22):
However, the sensitivity of such assays is limited by the number of labelled moieties which one may physically incorporate into the probe nucleic acid. In the case of radioactively-labelled probes, the practical limit of deletion is about 10.sup.4 target molecules. To achieve this sensitivity requires probes with radioactive labels which have a very high energy and a very limited useful lifetime. The detection step, autoradlography, requires several days. Other labelling methods utilizing fluorescent, chemiluminescent, or enzymatic detection, although more rapid, usually do not exceed the sensitivity of radioactively-labelled probes. Since most organisms of clinical interest do not contain more than 50,000 copies of any nucleic acid suitable for use as a target, the utility of such methods is restricted to the detection of large numbers of organisms. The level of infectious agents in clinical specimens or foodstuffs, however, often does not exceed one to ten organisms.

Brief Summary Text (23):
One approach for the detection of low levels of DNA utilizes a DNA-dependent DNA polymerase to directly replicate the DNA target to increase its numbers to easily detectable levels. This approach is termed "polymerase chain reaction" (PCR). Saiki, R. K., Scharf, S., Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H. A., and Arnheim, N., "Enzymatic Amplification of Beta-globin Genomic Sequences and

Restriction Site Analysis for Diagnosis of Sickle Cell Anemia," Science 230:1350-1354 (1985); Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B., and Erlich, H. A., "Primer-directed Enzymatic Amplification of DNA with a Thermostable DNA Polymerase," Science 239:487-491 (1988); Erlich, H. A., Gelfand, D. H., and Salki, R. K., "Specific DNA Amplification": Nature 331:461- (1988) and Mullis et al., European Patent application Nos. 200362 and 201184 (see also U.S. Pat. Nos. 4,683,195 and 4,683,202).

Brief Summary Text (24):
In practice, PCR is limited by the requirement that the target for amplification be DNA (as opposed to RNA), and by the occurrence of false positives generated by hybridization of probes to homologous sites in non-target DNA which fortuitously generate similar replication products. Moreover, although target DNA may be detected with very high sensitivity, the numbers of targets present in the sample is difficult to determine without adding significantly to the complexity of the assay. Since the number of infectious agents is often important in evaluating the treatment protocol for disease, this amplification approach is disadvantageously limited because it provides qualitative rather than quantitative results.

Brief Summary Text (26):
An autocatalytically replicatable RNA-probe construct may be employed in a sandwich hybridization assay, such as that described by Ranki, et al.. U.S. Pat. No. 4,563,419; Soderlund, G. B., U.S. Pat. No. 2,169,403; Stabinsky, U.S. Pat. No. 4,751,177; and Syvanenen, et al.., Nucl. Acids Res. 14:5037-5048. In the event the target is present and probe has hybridized to target, the autocatalytically replicatable RNA associated with the probe is replicated to generate amounts of RNA which may be easily detected by a variety of means (for example, by fluorescence using a dye such as ethidium bromide or propidium iodide). Since the MDV-1 RNA template for Q.beta. replicase is doubled in number every 20 seconds in vitro, an exponential increase (estimated to be a billion-fold) in the number of RNA molecules occurs within a few minutes at a single temperature. The autocatalytic reaction proceeds at an exponential rate until the number of autocatalytically replicatable RNA molecules exceeds the number of active enzyme molecules in the reactions. After that point, the amount of autocatalytically replicatable RNA increases linearly with time. As a consequence, in reactions given a sufficient period of time to reach this linear phase (for example 15 minutes for 100 molecules), the amount of amplified product RNA will be directly related to the logarithm of the number of autocatalytically replicatable RNAs initially added (Lizardi et al., supra). Since the initial number of autocatalytically replicatable RNA probes is proportional to the amount of target, the amount of target present in the sample being examined may be quantitated over a very wide range.

Brief Summary Text (28):
In another approach, the probe sequence may be incorporated within the sequence of the replicatable RNA (Lizardi et al., supra). However, the probe sequence is viewed as foreign by the enzyme and affects the ability of the RNA to be efficiently replicated, or is spontaneously deleted during replication. Deletion events affect the rate of replication and occur randomly with time. When deletion events occur, the level of the RNA products obtained in the linear phase of the amplification cannot be used to assess target level.

Brief Summary Text (30):
The discussion thus far has focused on signal generation. Signal generation which is related to the presence of target is very desirable. Signal generation which is not related to target, referred to as background, is undesirable. By way of example, a single autocatalytically replicatable RNA molecule in the presence of Q-Beta replicase and reaction conditions, will initiate the production of copies at an exponential rate. In the event such single autocatalytically replicatable RNA is associated with a probe, which probe is bound to target, the exponential replication is a true positive detection. In the event such single autocatalytically replicatable RNA is not associated with a probe, or if associated with a probe and such probe is not associated with target, the exponential replication is a false positive or constitutes background from which true signal must be differentiated. The presence of background limits the sensitivity of assays at low target

concentrations. Target induced signal must be significantly greater than background in order for assays to be considered reliable.

Brief Summary Text (31):
One form of background, in affinity assays, occurs when the probe having a label associates with molecules other than target, and is carried through to detection. This type of background is often associated with non-specific binding of probe to supports.

Brief Summary Text (32):
One approach to reducing this non-specific binding background employs a method by which the target-probe complex is reversibly bound to the support ("reversible target capture"). After hybridization and immobilization, the complex is eluted from the support, which is then discarded with the non-specifically bound probe. The target-probe is then recaptured on fresh support. This process may be repeated several times to produce a significant reduction in the amount of non-hybridized probe (see Collins, European Patent Application No. 87309308.2).

Brief Summary Text (37):
The present invention features means for the control and amplification of autocatalytically replicatable molecules for diagnostic and therapeutic purposes. One embodiment of the present invention features a composition of matter. The composition of matter comprises a first nucleic acid having a first section and a second section. The first section is capable of autocatalytic replication under reaction conditions as part of the first nucleic acid, which includes the second section. The second section, positioned at one of the ends of the first section, is capable of assuming a bound position with a target.

Brief Summary Text (40):
A further embodiment of the present invention features a first nucleic acid having an inhibitory element and a first section, and a second section. The first section is capable of active autocatalytic replication under reaction conditions when the first section is separated from the inhibitory element and is inactive when such first section is part of the first nucleic acid integral with the inhibitory element. The second section has sequences which are capable of interacting with release means to separate the first section from the inhibitory element. The first nucleic acid is capable of assuming a bound position with a target, in which said second section is capable of interacting with release means.

Brief Summary Text (43):
A further embodiment features-ligand and antiligand systems as inhibitory elements for inactivating the first section of the first nucleic acid. By way of example, ligands which are capable of binding to or interacting with the first section of the first nucleic acid may be capable of rendering the first section incapable of autocatalytic replication. Inhibitory elements utilizing ligand systems allow activation to occur following a cleavage event as a result of the destablization of the binding of the ligand system to the MDV-1 like sequences or activation may be due to the release of the first section to the extent that it is able to assume an active tertiary structure, or activation may be due to interactions with the enzyme. Inhibitory elements for inactivating the first section may include ligands and antiligand systems such as biotin-avidin or complementary nucleic acid sequences positioned in cooperating relationship on the first nucleic acid, antibody-antigen interactions and protein binding interactions.

Brief Summary Text (44):
One embodiment of the present invention features nucleotide sequences which are capable of interacting with the first section, rendering the first section inactive. One embodiment features sequences capable of interacting with MDV-1 like sequences in the first section in approximately the 81 to 126 MDV-1 nucleotide region. The interaction may include binding directly to the region but is not necessarily limited to such binding. The interaction may also include shielding the region from Interaction with the enzyme Q-beta replicase, interfering with the enzyme, and distorting the tertiary structure of the region. A preferred sequence of nucleotides Includes the nucleotides 5'-UUYRC-3'(SEQ ID NO:1), where Y represents any pyrimidine nucleotide and R is any purine nucleotide. One embodiment features inhibitory

sequences wherein Y is U and R is A.

Brief Summary Text (46):
In the specific case where the target is RNA, release means may be a small DNA oligonucleotide (for example, six nucleotides) complementary to a portion of the second section sequence of the first nucleic acid. In this case, the cleavage is ideally effected by the addition of RNase H (which acts to cleave RNA in RNA:DNA heteroduplexes) to the solution in contact with the support bearing the complex. Naturally, the means for capturing the RNA target should ideally avoid generation of such heteroduplexes in order for the cleavage event to be specific. For example, a biotinylated RNA complementary to another portion of the target RNA may be conveniently captured upon an immobilized streptavidin support.

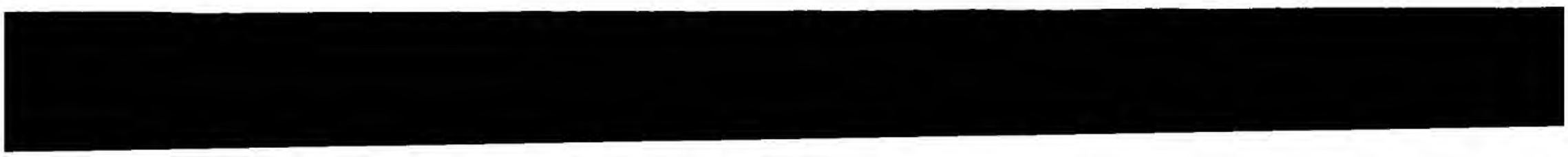
Brief Summary Text (47):
In the specific case where the target is DNA, digestion of the sequence extension of hybrids with target may be effected directly by the addition of RNase H, without the requirement for a second probe bearing such an oligonucleotide.

Brief Summary Text (50):
In one embodiment, the fourth section and second section require the presence of target which contributes nucleic acid sequences to form the ribozyme. The requirement for specific sequences in the target to form a ribozyme facilitates a further reduction in background. Signal can not be generated without target.

Brief Summary Text (51):
One such structure is described by the Formula I below: ##STR1## As used above, the letter X generally represents target, and X.sup.1 represents a first target region having one or more nucleotides which form a ribozyme with the first nucleic acid, X.sup.2 represents a terminal nucleotide of X.sup.1 or a second target region and X.sup.3 represents a terminal nucleotide of X.sup.1 or a third target region. The letter P generally represents the first nucleic acid, and P.sup.1 represents a first section, which section is capable of active autocatalytic replication when the first section is separated from an inhibitory element and inactive when the inhibitory element is integral with the first nucleic acid, in the presence of autocatalytic reaction conditions. The letter P.sup.2 represents a second section of first nucleic acid, which section contributes one or more nucleotides to form a ribozyme. The letter P.sup.3 represents an inhibitory element associated with the first nucleic acid. The letter P.sup.4 represents a fourth section capable of contributing one or more nucleotides to form a ribozyme.

Brief Summary Text (52):
In one embodiment which features a structure which resembles a "hammerhead" ribozyme, X.sup.1 and P.sup.4 are mutually exclusive and comprise one of the group of sequences 5'-MGAAAK-3'(SEQ ID NO:2), and 5'-J'CUGANGAM'-3'(SEQ ID NO:3), P.sup.2 comprises the sequences 5'-K'UWJ-3'(SEQ ID NO: 4), wherein the letter N represents a nucleotide selected from the group of nucleotides comprising A, G, U and C. The letter W represents C or A. The letters J, J', K, K', M and M' each represent four or more nucleotides. The nucleotides of J and J', are complementary, as are the nucleotides of K and K' and the nucleotides of M and M'. Such complementarity is believed to provide stability and alignments for the ribozyme structure. Complementarity among nucleotide groups within sections and areas of the same nucleic acid, such as K' and J of second section P.sup.2, J' and M' of fourth section P.sup.4, allows such sections to form "stem" loops which open only on interaction with target, rendering such first nucleic acid incapable of forming ribozyme structures without specific target interaction. Such groups of nucleotides may also incorporate inhibitory sequences which interact with the first section.

Brief Summary Text (53):
The inhibitory element, P.sup.3, can be any moiety capable of inhibiting autocatalytic replication. Preferably, the inhibitory element is a nucleic acid. In which case, P.sup.3 can be any sequence of nucleotides. However, for diagnostic and therapeutic purposes, it is useful to have greater specificity to target than the sequences of M, M', K and K' may provide. The inhibitory sequences can be sequences capable of assuming a bound position to target at target region X.sup.1.



Brief Summary Text (54):
A further embodiment of the present invention features a first nucleic acid and a second nucleic acid which in the bound position to target form a ribozyme. One such structure is described by the Formula II below: ##STR2## As used above, the letter X generally represents target, X.sup.1 represents a first target region, and X.sup.2 represents a terminal nucleotide of X.sup.1 or a second target region, and X.sup.3 represents a terminal nucleotide of X.sup.1 or a third target region. The letter P generally represents the first nucleic acid, P.sup.1 represents the first section which section is capable of active autocatalytic replication when the first section is separated from the inhibitory element and inactive when the inhibitory element is integral with the first nucleic acid and first section, in the presence of autocatalytic reaction conditions. The letter P.sup.2 represents a second section having one or more nucleotides which are capable of participating ribozyme formation. The letter P.sup.3 represents an inhibitory element. The letter P.sup.4 represents a terminal nucleotide of P.sup.1 or a fourth section capable of contributing sequences which participate in ribozyme formation with P.sup.2 and R.sup.1. As used above, the letter R generally represents the second nucleic acid, and R.sup.1 represents a first area capable of having one or more nucleotides which participate in ribozyme formation. The letter R.sup.2 represents a terminal nucleotide of R.sup.1 or represents a second area of the second nucleic acid capable of assuming a bound position with respect to target region X.sup.1. At least one of R.sup.1, R.sup.2, P.sup.2, P.sup.3, are capable of assuming a bound position with target.

Brief Summary Text (55):
In one embodiment, the structure formed resembles a "hammerhead" ribozyme. In which case, the letter P.sup.2 represents the sequence, 5'-K'UWJ-3' (SEQ ID NO:4), X.sup.1 and R.sup.1 are mutually exclusive and represent one of the group of sequences 5'-MGAAAK-3' (SEQ ID NO:2), and 5'-J'CUGANGAM'-3' (SEQ ID NO: 3), wherein N is one of the nucleotides U, G. A and C. The letter W represents C or A. The letters J, J', K, K', M and M' each represent four or more nucleotides. The nucleotides of J and J' are complementary as are the nucleotides of K and K' and the nucleotides of M and M'. P.sup.4 is a terminal nucleotide of P.sup.1.

Brief Summary Text (56):
Complementarity between nucleotide groups within an area or section, such as K' and J of second section P.sup.2, and J' and M' of first area R', allows such sections and areas to form "stem" loops which open up only on interaction with target, rendering such first nucleic acid incapable of forming a ribozyme structure without specific target interaction. Such areas may also incorporate inhibitory sequences which interact with the first section.

Brief Summary Text (58):
In one embodiment, the structure formed resembles a "hairpin" ribozyme. In which case, P.sup.2 represents the sequences 5'-FNGUCQ-3' (SEQ ID NO:5). The area represented by R.sup.1 comprises the sequences 5'-Q'AGAAF'ACCAGAGAAACACACGUUGUGGUAUAUUACCUGGUA-3' (SEQ ID NO:6). At least one of R.sup.2 and P.sup.3 are capable of assuming a bound position to target at X.sup.1, X.sup.2 or X.sup.3. The letter Q and Q', F and F' each represent four or more nucleotides. The letter N represents one of the nucleotides U, G, A, C. The nucleotides of Q and Q' are complementary, as are the nucleotides of F and F'. At least one of P.sup.3 or R.sup.2 are capable of assuming a bound position to target. The inhibitory element binding at target region X.sup.3 and the second area of the second nucleic acid assuming a bound position at target region X.sup.2.

Brief Summary Text (59):
Complementarity between nucleotide groups within an area or section, such as F and Q of second section P.sup.2, and F' and Q' of first area R', allows such sections and areas to form "stem" loops which open up only on interacting with target, rendering such first and second nucleic acids incapable of forming a ribozyme without specific target interaction. Such areas may also incorporate inhibitory sequences which interact with the first section.

Brief Summary Text (60):
As described with respect to Formula I, and now with respect to Formula II, greater

specificity is obtained for target where the inhibitory element P.sup.3 is capable of assuming a bound position with target. In systems described with respect to Formula II, utilizing a first nucleic acid and a second nucleic acid, greater specificity can be obtained where R.sup.2 is capable of assuming a bound position with target.

Brief Summary Text (61):
The characteristic inability of the autocatalytically replicatable sequences to replicate efficiently until associated probes are specifically bound to target has led to descriptions of such compositions, as applied in diagnostics, as "smart probes." Smart probes provide reduction in background because they produce less or no signal until bound to target.

Brief Summary Text (62):
Compositions of the present invention have features of smart probes. Such smart probes have application in diagnostics, and can be used in conjunction with a variety of methods for background reduction such as sandwich assays and reversible target capture. However, the inherent low background of the compositions of the present invention may allow the compositions to be used in systems without a sandwich format and without reversible target capture.

Brief Summary Text (63):
One composition of the present invention features a first nucleic acid having a first section, and a second section, and inhibitory element and a support means. Each section has a 5' end and a 3' end. The support means is capable of binding to a support or is associated with a support. The first section is capable of active autocatalytic replication when the first section is separated from the inhibitory element and is inactive when the inhibitory element is associated with the first section. The inhibitory element is associated with the first section through the second section. The second section is capable of forming a ribozyme in the presence of target, which ribozyme cleaves the first section from the ligand under ribozyme reaction conditions.

Brief Summary Text (64):
An embodiment of the composition is described by Formula III below: ##STR3## As used above, the letter X generally represents target, X.sup.1 represents a first region, and X.sup.2 represents the terminal nucleotide of X.sup.1 or X.sup.2 represents a second region, X.sup.3 represents the terminal nucleotide of X.sup.1 or represents a third target region. The letter P generally represents a first nucleic acid, P.sup.1 represents a first section capable of active autocatalytic replication when the first section is separated from the inhibitory element, and inactive when integral with the inhibitory element. The letter P.sup.2 represents a second section capable of forming a ribozyme in the presence of the target. The letter P.sup.3 represents an inhibitory element such as, without limitation, nucleotide sequences capable of interaction with the first section, or such nucleotides which by virtue of their number, inhibit autocatalytic replication. The letter P.sup.4 represents the terminal nucleotide of the first section P.sup.1, or represents a fourth section-capable of participating in the formation of a ribozyme. Support means, generally represented by the letter S, is any conventional or retrievable support, or a ligand capable of binding to such support. Support means may constitute inhibitory element, or inhibitory element may be part of support means.

Brief Summary Text (65):
One embodiment, which features a ribozyme which resembles "hammerhead" structure, P.sup.2 represents the sequences 5'-K'UWJ-3' (SEQ ID NO:4), X.sup.1 and P.sup.4 are mutually exclusive and are selected from the groups of sequences 5'-MGAAAK-3' (SEQ ID NO:2) and 5'-J'CUGANGAM'-3' (SEQ ID NO:3), wherein N is one of the bases U, G, A, and C. The letter W represents C or A. The letters J, J', K, K', M and M' each represent four or more nucleotides. The nucleotides of J and J' are complementary, as are the nucleotides of K and K', and the nucleotides of M and M'.

Brief Summary Text (66):
The composition may also comprise a second nucleic acid which cooperates with the first nucleic acid to form a ribozyme in the presence of target. Such a composition is described by the Formula IV set forth below: ##STR4## As used above, the letters

X.sup.ì, X.sup.2, X.sup.3, S, P.sup.4, P.sup.3, P.sup.2 and P.sup.1 are consistent with the description immediately above. The letter R generally represents a second nucleic acid having a first area and a second area. The first area represented by the letter R.sup.1, is capable of forming a ribozyme with the second section P.sup.2 of the first nucleic acid. The letter P.sup.4 represents the terminal nucleotide of P.sup.1 or represents a fourth section to which contributes sequences to form a ribozyme. The second section is represented by the letter R.sup.2, is the terminal nucleotide of R.sup.1 or represents an area of the second nucleic acid capable assuming a bound position to the target at X.sup.3.

Brief Summary Text (67):
In one embodiment which resembles a "hairpin" ribozyme, P.sup.2 represents the nucleotide sequences 5'-FNGUCQ-3' (SEQ ID NO:5), R represents the nucleotide sequences 5'-Q'AGAAF'ACCAGAGAAACACACGUUGUGGUAUAUUACC UGGUA-3' (SEQ ID NO:6). The letters Q, Q', F and F' each represent four or more nucleotides. The nucleotides of Q and Q' are complementary as are the nucleotides of F and F'. P.sup.4 is the terminal nucleotide of P.sup.1. At least one of the sections or areas of the first nucleic acid and second nucleic acid are capable of assuming a bound position. The second area of the second nucleic acid assuming a bound position at target third region X.sup.3 and inhibitory element P.sup.3 assuming a bound position with target at target second region X.sup.2.

Brief Summary Text (68):
In one embodiment, which features a structure which resembles a "hammerhead" ribozyme, P.sup.2 represents the sequences 5'-K'UWJ-3' (SEQ ID NO:4), X.sup.1 and R.sup.1 are mutually exclusive and represent one of the groups of sequences 5'-MGAAAK-3' (SEQ ID NO:2) and 5'-J'CUGANGAM'-3-40 (SEQ ID NO:3), wherein N is one of the bases U, G, A and C. The letter N represents C or A. The letters J, J', K, K', M and M' each represent four nucleotides. The nucleotides of J and J' are complementary, as are the nucleotides of K and K' and the nucleotides of M and M'. P.sup.4 is the terminal nucleotide of P.sup.1.

Brief Summary Text (70):
The support means in formulas III and IV allows the target nucleic acid complex to be separated from debris, reagents, and other nucleic acid which may be present in the sample. The first section of the first nucleic acid is inactive, unless in the presence of target it is separated or cleaved, minimizing or eliminating background.

Brief Summary Text (71):
By way of example, support means may include a biotin group for capture upon a support derivatized with avidin or streptavidin, a fluorescein group for capture upon a support bearing immobilized antibodies to fluorescein, a poly A tail for capture upon a support bearing immobilized oligo or poly dT and a binding site for the coat protein of bacteriophage R17 for capture upon a support bearing the coat protein.

Brief Summary Text (72):
A number of means may be employed to associate a ligand with the first nucleic acid. By way of example, where the support means is 3' to the site of cleavage, biotin, fluorescein, proteins, antibodies and antigens may be associated by one of several methods. These include, but are not limited to: (1) ligating a small RNA or DNA oligonucleotide produced synthetically and containing one or more biotins to the 3' terminus with T4 RNA ligase (2) addition of an RNA tail to the 3' terminus with E. coli poly A polymerase using biotinylated ribonucleoside triphosphates (3) periodate oxidation of the 3' terminal residue, followed by coupling of the dialdehyde product to a biotinylated molecule bearing a primary amine followed by reduction, and (4) hybridization of a biotinylated complementary RNA to a region distal (e.g.-3' to) the target-binding region of the midivariant probe.

Brief Summary Text (73):
Turning now tò methods of the present invention which relate to diagnostics, the methods of the present invention feature each of the composition herein described. This discussion will focus on selected compositions by way of example, without limitation. One embodiment of the present invention includes a method for detecting

the presence of a target nucleic acid in a sample comprising the steps of contacting a sample with a first nucleic acid which nucleic acid has a first section and a second section. The first section is capable of autocatalytic replication under reaction conditions. The first section has a 3' end and a 5' end. The second section is positioned at one of the ends of the first section and is capable of assuming a bound position in the presence of binding conditions with target. The method further includes the step of imposing binding conditions on the sample to allow the first nucleic acid to form a target-first nucleic acid complex. Unbound first nucleic acid is separated from the sample. The imposition of reaction conditions for autocatalytic replication on the sample allows the sample to be monitored for the presence of the autocatalytic reaction product which will be formed in the presence of the first nucleic acid, which reaction product is indicative of the presence of the target.

Brief Summary Text (74):
A further embodiment of the present invention includes a first section which has sequences which are substantially identical to MDV-1 and is capable of autocatalytic replication in the presence of the enzyme Q-beta replicase. Preferably, in imposing reaction conditions, which reaction conditions Include contacting the sample with the enzyme Q-beta replicase, an enzyme is used which has no endogenous activity. Surprisingly and unexpectedly, when such nucleic acid compositions with first sections having sequences substantially identical to MDV-1, and second sections capable of binding to target, are used with the enzyme Q-beta replicase, it is possible to discern the presence of target from what had been previously characterized as "unprimed" activity.

Brief Summary Text (75):
A further embodiment includes a method for detecting the presence of target nucleic acid in a sample comprising the step of contacting the sample with a first nucleic acid which nucleic acid has a first section, and second section and is associated with an inhibitory element. Each section has a 5' end and a 3' end. The first section is capable of active autocatalytic replication under reaction conditions when the first section is separated from the inhibitory element and is inactive when integral and associated with the inhibitory element. The second section is positioned at one of the ends of the first section and is capable of cleavage upon interaction with release means, to separate the first section from the inhibitory element. At least one section or the inhibitory element of the first nucleic acid is capable of assuming a bound position to target in the presence of binding conditions. Imposition of binding conditions on the sample in the first nucleic acid allows the formation of a target first nucleic acid complex in the presence of target. The sample is contacted with release means to separate the first section from the inhibitory element. Imposition of autocatalytic replication reaction conditions on the sample, allows the formation of an autocatalytic reaction product in the presence of the first nucleic acid. The sample is monitored for the presence of the autocatalytic reaction product, which autocatalytic reaction product is indicative of presence of target.

Brief Summary Text (78):
One embodiment of the present Invention features a ribozyme formed by the first nucleic acid and the target. The method incorporates additional "smartness" due to the need for specific target sequences which do not allow a ribozyme to form unless the first nucleic acid is bound to target. One embodiment features a ribozyme which requires target sequences to form the ribozyme.

Brief Summary Text (79):
In a further embodiment, the method includes the formation of a ribozyme comprising a first nucleic acid and a second nucleic acid, both of which can be made to interact with target in order to form stable ribozyme structures.

Brief Summary Text (80):
In one structure resembling a "hammerhead," the target contributes the sequences 5'-MGAAAK-3' (SEQ ID NO:2), a first nucleic acid or second nucleic acid has a section or an area which contributes the sequences 5'-J'CUGANGAM'-3' (SEQ ID NO:3), and first nucleic acid has a section which contributes the sequences 5'-K'UWJ-3' (SEQ ID NO:4). As used herein, N is one of the four nucleotides U, G, A and C. The

letter W represents C or A. The letters J, J', K, K', M and M' each represent four or more nucleotides. The nucleotides of J and J' are complementary, as are the nucleotides of K and K' and the nucleotides of M and M'.

Brief Summary Text (83):
Additional embodiments of the present invention relate to compositions of matter which are useful as ribozymes. One embodiment of the present invention features a first nucleic acid and a second nucleic acid in which the first nucleic acid has a first section and a second section. Each section having a 5' end and a 3' end. The first section has less than all the sequences performing a ribozyme and the second section is capable of assuming a bound position with a target and is positioned at one of the ends of the first section. The second nucleic acid has a first area and a second area, each area having a 5' end and a 3' end. The first area has sequences which complete the formation of a ribozyme when the first area is held in a cooperating relationship with the first section of the first nucleic acid. The second area is capable of assuming a bound position to target in which the first area is held in cooperating relationship.

Brief Summary Text (84):
In one embodiment of the present invention, the ribozyme resembles a "hammerhead." In one "hammerhead" configuration, the target contributes the sequences 5'-MGAAAK-3' (SEQ ID NO:2) for the completion of the ribozyme. The first section of the first nucleic acid includes the sequences 5'-J'CUGANGAM'-3' (SEQ ID NO:3) wherein the letter N is used to represent a nucleotide selected from the group of nucleotides consisting of A, G, U or C. The first area of the second nucleic acid contributes sequences 5'-K'UWJ-3' (SEQ ID NO:4). The letter W represents C or A. The letters J, J', K, K', M, and M' each represent four or more nucleotides. The nucleotides of J and J' are complementary, as are the nucleotides of K and K', and the nucleotides of M and M'.

Brief Summary Text (86):
Further embodiments of the present invention feature methods of making ribozymes. In one embodiment, the method includes binding a first and second nucleic acid to a target in cooperating relationship. The first nucleic acid has less than all sequences for ribozyme activity. The second nucleic acid has less than all sequences necessary for all ribozyme activity and in a bound position, form a ribozyme.

Brief Summary Text (88):
In order to facilitate an understanding of the invention, the discussion with respect to ribozyme compositions and methods of making ribozymes has focused on nucleic acid probes. However, sections and areas of the first and second nucleic acid which bind to a target can be substituted with any ligand which binds to a target to position sections and areas which form a catalytic sequence group. Embodiments of the present invention featuring the formation of ribozymes encompass the use of probes in a broad sense including immunodiagnostic agents, such as antibodies and antigens, and other biological binding pairs such as, drugs and drug receptor sites, enzymes and enzyme substrates.

Drawing Description Text (2):
FIG. 1, consisting of FIG. 1A and FIG. 1B, illustrates a method of determining the presence of a target molecule which target molecule is capable of contributing sequences to form a ribozyme in a "hammerhead" configuration.

Drawing Description Text (3):
FIG. 2 generally depicts an alternative "hammerhead" ribozyme configuration.

Drawing Description Text (4):
FIG. 3 generally illustrates a first and second nucleic acid bound to target forming a ribozyme in a "hairpin" configuration.

Drawing Description Text (5):
FIG. 4 illustrates a structure of a ribozyme with an autocatalytic replicating section associated with a support, in the presence of target.

Detailed Description Text (2):

To facilitate discussion of the present invention, reference will be made to the figures which describe methods and compositions for the detection of target. However, those skilled in the art will readily recognize that the methods and compositions of the present invention have value and application in therapeutics to deliver molecules to sites for activation.

Detailed Description Text (3):
Turning now to FIG. 1, which consists of FIGS. 1A and 1B, a method of detecting the presence of a target molecule is generally depicted.

Detailed Description Text (4):
As illustrated, the target molecule is a nucleic acid generally designated by the number 11. The target molecule is associated with a capture bead 13 by means of a capture ligand 15 which is hybridized to a capture nucleic acid, generally described by numeral 21. The capture nucleic acid 21 has a capture antiligand section generally described by the numeral 23 and a probe section 25 capable of hybridizing to the target 11. The capture nucleic acid is illustrated in a hybridized position to the target 11.

Detailed Description Text (5):
The target 11 has a first segment 26 and a second segment 27 and a third section 29. The first segment 26 and third segment 29 define binding sites. The second segment 27 has sequences which participate in the formation of a ribozyme.

Detailed Description Text (6):
A first nucleic acid 31 is illustrated bound to the target 11. The first nucleic acid 31 has a first section 33, second section 35, a third section 37 (as can be seen in FIGS. 2, 3, and 4) and a fourth section 41. The first section 33 is capable of autocatalytic replication. As illustrated, the sequences of the first section 33 includes sequences that are substantially identical to the sequences of MDV-1. The second section 35 has sequences which are able to participate in the formation of a ribozyme. The third section 37 is capable of binding to the target at the target segment 27. The fourth section 41 includes at least four base sequences 43 capable of binding the target at target third segment 29, and contributes sequences to the formation of the ribozyme. The sequences of the fourth section 41 which participate in ribozyme formation are generally designated by the numeral 45 and those sequences capable of binding target are designated 43. Upon imposition of ribozyme reaction conditions in the presence and target, the first nucleic acid 31 is capable of cleavage in second section 35 (see FIGS. 2 and 3) at the position designated as 39.

Detailed Description Text (8):
Thus, in FIG. 1B, the first section 33 is shown as a distinct part from the rest of the first nucleic acid 31. The few remaining nucleotides of the fourth section 41 which are capable of binding to the second nucleic acid at 29, are unable to form a stable hybrid under the conditions present in the sample. The first section 33 becomes disassociated from the target-probe complex and receptive to autocatalytic replication reaction conditions.

Detailed Description Text (9):
Upon imposition of autocatalytic reaction conditions, the first section 33 is replicated on an exponential basis initially. The fourth section 41 need not, and normally is not, replicated. Upon saturation of the enzyme Q-beta replicase with the first section 33 and its copies, the first section 33 and its copies are replicated in a linear fashion. In a diagnostic assay, the sample is monitored for the presence of the autocatalytic reaction product which product is indicative of the presence of target 11. Additionally, if one monitors the concentration of the autocatalytic reaction product, the concentration of target 11 can be calculated on the basis of the amount of autocatalytic reaction product produced in time.

Detailed Description Text (10):
FIG. 2 describes an alternative "hammerhead" ribozyme structure formed by target 11, and a first nucleic acid 31 and a second nucleic acid 41. The ribozyme structure depicted in FIG. 2 has a cleavage site 39 is positioned towards the 5' end of the first nucleic acid 31.

[redacted]

Detailed Description Text (11):
The target 11 includes a first segment 25, second segment 27 and a third segment 29. A first nucleic acid 31 includes a first section 33, a second section 35 and a third section 37. The first section includes base sequences which are substantially identical to MDV-1 and are capable of autocatalytic replication when removed from the third section 37. The second section 35 is capable of contributing sequences which form a ribozyme. The third section 37 is capable of binding to the target 11 at the third segment 29.

Detailed Description Text (12):
A second nucleic acid 41 includes a first area 43 and a second area 45. The first area 43 is capable of binding to the target 11 at first segment 25. The second area 45 of the second nucleic acid 41 is capable of contributing sequences to form a ribozyme.

Detailed Description Text (13):
As illustrated, the target 11 itself contributes sequences to the ribozyme, which sequences include the sequences 5'-GAAA-3' (SEQ ID NO:7). Individuals skilled in the art will recognize that the designation of target 11 and second nucleic acid 41 is somewhat arbitrary, depending on the molecule of interest and what sequences are available in such molecule. It is much more probable that a particular molecule of interest will have the sequences 5'-GAAA-3' (SEQ ID NO:7), than the sequences 5'-CUGANGA-3' (SEQ ID NO:8). In a bound position to the target 11 the first nucleic acid 31 and the second nucleic acid 41 form a ribozyme which upon imposition of ribozyme reaction conditions cause cleavage at position 39, releasing the first section 33 from the third section 37. The first section 33, removed from the third section 37 is capable of autocatalytic replication upon imposition of autocatalytic replication reaction conditions.

Detailed Description Text (14):
Turning now to FIG. 3, a target is illustrated generally depicted as 11 in which a first nucleic acid 31 and a second nucleic acid 41 are bound. The first nucleic acid 31 and the second nucleic acid 41 create a "hairpin" ribozyme structure. The target 11 includes a first segment 27 and a second segment 29 each having a 5' end and a 3' end. The first nucleic acid 31 includes a first section 33, a second section 35 and a third section 37. The first section 33 is capable of autocatalytic replication in the presence of autocatalytic reaction conditions and upon separation from the third section. The second area 35 is capable of contributing sequences to a ribozyme structure. The third section 37 is capable of binding to the first segment 27 of the target 11.

Detailed Description Text (16):
To facilitate synthesis and cloning manufacturing, each area of the second nucleic acid 41 is linked by a conventional phosphodiester bridge 5' to 3' or 3' to 5'. The first area 43 is capable of assuming a bound position to the second segment 29 of the target 11. The second area 45 of the second nucleic acid 41 is capable of forming a "hairpin" ribozyme with the second section 35 of the first nucleic acid 31. The second area 45 of the second nucleic acid 41 and the second section 35 of the first nucleic acid 31 define a ribozyme having a cleavage site 39 on the first nucleic acid 31.

Detailed Description Text (18):
FIG. 4 illustrates compositions similar to that illustrated in FIG. 3. A target is illustrated generally depicted as 11 in which a first nucleic acid 31 and a second nucleic acid 41 are bound. The first nucleic acid 31 and the second nucleic acid 41 create a "hairpin" ribozyme structure. The target 11 includes a first segment 27 and a second segment 29 each having a 5' end and a 3' end. The first nucleic acid 31 has a first section 33, a second section 35 and a third section 37. The first section 33 is capable of autocatalytic replication in the presence of autocatalytic reaction conditions and upon separation from the third section. The second area 35 capable of contributing sequences to a ribozyme structure. The third section 37 is capable of binding to the first segment 27 of the target 11, and Is associated with support means 51. Support means 51 is biotin which is capable of binding to avidin 53 bound to support 13.

[redacted]

Detailed Description Text (20):
To facilitate synthesis and cloning manufacturing, each area of the second nucleic acid 41 is linked by a conventional phosphodiester bridge 5' to 3' or 3' to 5'. The first area 43 is capable of assuming a bound position to the second segment 29 of the target 11. The second area 45 of the second nucleic acid 41 is capable of forming a "hairpin" ribozyme with the second section 35 of the first nucleic acid 31. The second area 45 of the second nucleic acid 41 and the second section 35 of the first nucleic acid 31 define a ribozyme having a cleavage site 39 on the first nucleic acid 31.

Detailed Description Text (21):
After a complex of the target 11 with the first nucleic acid 31 and second nucleic acid 41 is formed, the first nucleic acid 31 is captured on support 13 through the biotin 51 and avidin 53. The support 13 is separated from the remaining solutions, which may include first nucleic acid 31 unbound to target 11, and first sections 33 which have dissociated from the first nucleic acid 31 through an event not mediated by target 11, to reduce background.

Detailed Description Text (30):
Two oligonucleotides are synthesized. The first of these advantageously contains the sequence 5'-CCCCTGANGA-3' (SEQ ID NO:10) followed by at least four nucleotides complementary to the sequence in the target RNA 5' to the 5'-GAAA-3' (SEQ ID NO:7) element and terminating in the sequence 5'-GATC-3' (SEQ ID NO:11). The second oligonucleotide ideally contains the sequence 5'-CCCGA-3' (SEQ ID NO:12) followed by at least 4 nucleotides of the sequence 3' to the 5'-GAAA-3' (SEQ ID NO:7) element in the target, except that deoxyribonucleotides replace the ribonucleotides of the target. This element is advantageously followed Immediately by the element 5'-GGGG-3' (SEQ ID NO:13). Each of the oligonucleotides is phosphorylated on its 5' terminus by T4 polynucleotide kinase.

Detailed Description Text (36):
The cDNA clone as described in Example 1 is cleaved with the enzymes Apa I and Ear I, and the large fragment is ideally purified away from the small fragment described above. An oligonucleotide having the sequence 5'-CCCGA-3' (SEQ ID NO:12) followed by 4-50 nucleotides identical to the sequence immediately 3' to a 5'-GAAA-3' (SEQ ID NO:7) element in the target, in turn followed by the sequence 5'-GGCC-3' (SEQ ID NO:14) is synthesized by any of a number of methods familiar to those skilled in the art. This oligonucleotide is annealed and ligated to the large restriction fragment, and the resulting single stranded "gap" region rendered double-stranded by the action of a DNA-dependent DNA polymerase. This DNA is introduced into and propagated within bacteria. After purification from bacteria, it may be cleaved with the restriction endonuclease Sma I, and then used as a template in an in vitro transcription reaction utilizing bacteriophage T7 RNA polymerase.

Detailed Description Text (37):
The second sequence probe may be generated by constructing a synthetic DNA template for T7 RNA polymerase as described by Milligan et al. (Nucl. Acid. Res. (1987)15:8783-8798), one strand of this template starting at its 5' end with at least 4 nucleotides of the sequence from the region 5' to the 5'-GAAA-3' (SEQ ID NO:7) element in the target except that deoxyribonucleotides replace the ribonucleotides found in the target, followed by the sequence 5'-TCNTCAGGGGGCCCTATAGTGAGTCGTATTA-3' (SEQ ID NO:15) where N indicates any nucleotide having the sequence 5'-TAATACGACTCACTATAG-3' (SEQ ID NO:16). These two oligonucleotides preferably are mixed and transcribed in vitro as described by Milligan et al. (ibid). The product may be readily purified by any of a number of methods familiar to those acquainted with the art.

Detailed Description Text (43):
An assay for target nucleic acid utilizing a ribozyme probe.

Detailed Description Text (45):
One picomole (10.sup.-12 mol) of this RNA is hybridized to whole human blood from patients infected with human immunodeficiency virus type 1 (HIV-1), normal human blood, or a sample to be assayed for the presence of HIV-1, in a sandwich hybridization assay such as described by Ranki et al. (ibid) in the presence of 2.5M

guanidine thiocyanate and one picomole of a synthetic deoxyribo-oligonucleotide having the sequence 5'-GGAAGCACATTGTACTGATATCTAATCCCTGGTGGTCTCATA.sub.150 -3' (SEQ ID NO:21). With general reference to FIG. 1A the hybrid is bound to a solid support by hybridization of the dA150 tract in the oligonucleotide to immobilized polydeoxythymidine such as in the well of a polystyrene microtiter place (or a capture bead as shown in FIG. 1A) coated with dT.sub.3000 according to the methods of Collins et al., (U.S. Ser. No. 922,155 and CIP U.S. Ser. No. 136,920, fully incorporated herein by reference). The plate is incubated at 37.degree. C. for 30 minutes after which the contents, containing unbound probes, are aspirated and discarded, and the well repeatedly washed with a buffer containing 9.5M guanidine thiocyanate. The well is then washed with a buffer containing 90 mM Tris HCl (pH7.5), and 50 .mu.l of the same buffer except containing 14 mM MgCl.sub.2 introduced into the well and incubated at 50.degree. C. for 15 minutes to induce ribozyme cleavage. The contents of the well containing the cleaved and released probe (see FIG. 1B) are transferred to another well for replication. Five microliters of a solution containing 4 mM each ATP, GTP, CTP, and UTP, 10 .mu.lCi of .alpha.-.sup.32 P-CTP and 1 .mu.g of Q.beta. replicase are added and the plate incubated at 37.degree. C. for 25 minutes. Two microliters of the well contents are removed and added to 18 .mu.l of 95% formamide, 0.05% xylene cyanol, 0.05% bromphenol blue for halting replication. Five microliters of this are applied to the well of a denaturing 8% polyacrylamide gel, and the replicated RNAs resolved by electrophoresis until the xylene cyanol dye has migrated the length of the gel. An x-ray film is exposed to the gel for three hours and is then developed as the final step of detection. The larger RNA product of length equal to the number of nucleotides between the sequence corresponding to the unappended 5' terminus of the naturally-occurring MDV-1 RNA and the unappended 3' terminus of MDV-1 RNA and including the length of the sequence element inserted into the Hinf site such as described in Example 1 (in that case 10 nucleotides), indicates the presence of HIV-1 virus or its messenger RNA in the blood sample. It will also be readily recognized that by carefully timing the replication phase and comparing the results against a suitable control, correlation can be made between the amount of replicated RNA with the amount of target RNA in the original sample thereby providing quantitation results.

Detailed Description Text (48):
An assay for target HIV-1

Detailed Description Text (56):
These results provide practical guidance for using replicatable RNAs bearing 3' sequence extensions as probes for the sensitive detection of nucleic acids. By appropriate selection of additional sequences from the 3' end, RNA probes may be advantageously generated which have intrinsically greater sensitivity in the detection of target nucleic acid. Conversely, for some target nucleic acids which are present in an infectious agent at high levels (i.e.-ribosomal RNA, present at up to 50,000 copies per organism), additional sequences conferring relatively poor limits of detection may be utilized to advantageously avoid the background otherwise generated by the amplification of lower levels of non-specifically bound probes. For example, if reduced in number to levels at least an order of magnitude below the limit of detection using background reduction methods such as Collins (supra), non-specifically bound probes will be incapable of producing a detectable signal. Thus, the probes of the present invention comprising additional sequences from the 5' end may be used advantageously to reduce the cost, complexity, and frequency of false-positive reactions in such assays.

Detailed Description Text (59):
An important advantage issued from the present discovery which has additional application for smart probe systems which employ the target-directed cleavage of 5' or 3' sequence extensions, since such methods need not provide for the precise and complete removal of those sequences. For example and as set forth in U.S. Ser. No. 252,243, a probe may be generated bearing both 3' and 5' extensions whose hybridization to target produces a ribozyme structure which directs cleavage of the 5' extension from the RNA. A short 3' extension remains on the molecule.

Detailed Description Text (60):
A most preferred embodiment of the present invention is diagrammed in FIG. 4,

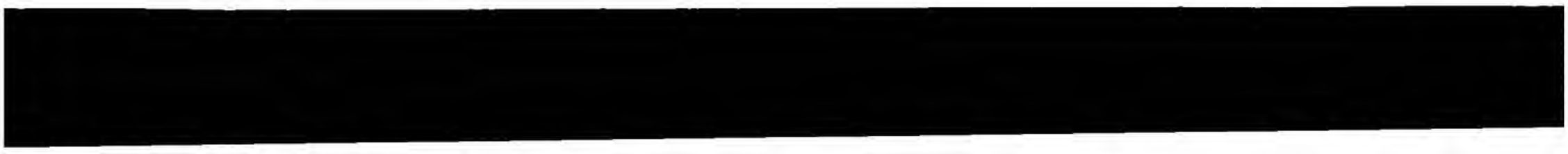
wherein a probe molecule bearing an extension is hybridized to target in solution. Shortly before, after, or simultaneously therewith, a second oligonucleotide probe is hybridized to the target adjacent to the first probe. The second oligonucleotide probe is ideally coupled to a nuclease which requires a cofactor (for example, a divalent cation) for its activity. Following hybridization, the complex is captured on a solid support in a manner such as that described by Rankmi et al. (supra), Soderlund (supra), Stabinsky (supra), and/or Syvanenen (supra). After separating, such as by washing away, the bulk of non-specifically bound probes, the cofactor for nuclease activity is added. The nuclease, coupled to the terminus of the probe proximal to the first probe when hybridized to its target, cleaves the spacer on the first probe, thereby releasing a replicatable moiety into solution. As previously indicated, the third section may be advantageously selected to maximally inhibit replication until cleavage occurs. Other variations of this approach will become apparent. For example, such probe parts may include a replicatable RNA instead bearing a 5' extension and a nuclease-coupled probe in which the ribozyme sequence is present 5' to the probe sequence element. Any of a number of nucleases may be advantageously employed as the release means. For example, micrococcal nuclease may be used since it does not cleave MDV-1 RNA (Hill & Blumenthal (1983) Nature 301, 350-352). Corey & Schultz (1987) Science 238:1401-1403, teach the construction of such oligonucleotide-micrococcal nuclease conjugates. Any number of ribozymes with endonucleolytic cleavage activity, such as those described by Haselhoff & Gerlach (Nature 3343:585-591 (1988)), Uhlenbeck (1987) Nature 328, 596-600, Ruffner (1990) Biochemistry 29,10695-10702, Cech (European pat. Appl. WO 88/04300, June 1988), Sharmeen et al. (1989) J. Virol. 63, 1428-1430; or Hampel & Tritz (1988) J. Cell. Biochem., Suppl. 12D, Abst. #N212, p.31, may be employed. Ribozymes possess a significant advantage in that the second probe bearing the ribozyme may be efficiently produced in a single step by transcription of a DNA oligonucleotide of appropriate sequence such as is described by Milligan (supra), thus reducing the cost and labor required to generate such reagents. Moreover, ribozymes tend to produce specific cleavage events, leading to a product RNA with defined replication properties.

Detailed Description Text (61):
In the specific case where the target is RNA, release means may be a small DNA oligonucleotide (for example, six nucleotides) complementary to a portion of the second sequence section of the first nucleic acid. In this case, the cleavage is ideally effected by the addition of RNase H (which acts to cleave RNA in RNA:DNA heteroduplexes) to the solution in contact with the support bearing the complex. Naturally, the means for capturing the RNA target should ideally avoid generation of such heteroduplexes in order for the cleavage event to be specific. For example, a biotinylated RNA complementary to another portion of the target RNA may be conveniently captured upon an immobilized streptavidin support.

Detailed Description Text (62):
In the specific case where the target is DNA, digestion of the sequence extension of hybrids with target may be effected directly by the addition of RNase H. without the requirement for a second probe bearing such an oligonucleotide.

Detailed Description Text (65):
Two oligonucleotides having the sequences: (1)
5'-AATTCTATGTGATATCAGCTAGTTGGTGGGGTAAAGGCCT-3' (SEQ ID NO:28); and (2)
5'-AATTAGGCCTTTACCCCACCAACTAGCTGATATCACATAG-3' (SEQ ID NO:29) were annealed and ligated into a MDV cDNA construct similar to that described by Lizardi (supra), which had been digested with EcoRI. This cDNA clone differed from those described by Lizardi et al. in that the internal insert encoded a binding site for the coat protein for phage R17. This cDNA was obtained from F. R. Kramer, Public Health Research Institute, N.Y. The oligonucleotides were ligated in the orientation such that upon subsequent digestion with EcoRI, cleavage occurred downstream of the oligonucleotide with respect to the promoter for T7 RNA polymerase. The digested plasmid was transcribed in vitro with T7 RNA polymerase under the conditions described by Mulligan et al. (supra), and the transcription product of correct length isolated by electrophoresis through polyacrylamide gels containing 8.3M urea.

Detailed Description Text (67):



Various numbers of formalin-fixed elementary bodies of Chlamydia trachomatis were lysed in a solution of 9.0 mg/ml proteinase K (Boehringer Mannheim) and 1.6% Sarkosine (Sigma) at 65.degree. C./for 15 minutes in a final volume of 35 .mu.l. Thirty-five .mu.l of a buffer containing 340 ng/ml of tailed capture oligonucleotide and 100 ng/ml of the transcription product probe was then added and solution phase hybridization allowed to occur for 30 minutes at 37.degree. C. Fifty .mu.l of a 0.06% (w/v) suspension of oligo-dT derivitized magnetic beads prepared according to Collins (supra) in 4% BSA, 10 mM EDTA, 0.2% Sarkosine, 9.1 M Tris-HCl pH8.0 and 0.05% bronopol was then added and incubated for an additional 5 minutes at 37.degree. C. to capture the target-probe hybrids on the beads.

Detailed Description Text (69):
The collected beads, freed of supernatant, were then resuspended in 50 .mu.l of a buffer containing 3.25M GuSCN, 65 mM EDTA, 0.04M Tris-HCl, pH7.0, 0.5% Sarkosine, and 0.5% BSA, and incubated at 37.degree. C. for 5 minutes to release the target-MDV probe-capture probe hybrids. The magnetic beads were collected as before, and the supernatants removed and transferred to a fresh set of tubes, each containing 50 .mu.l of a fresh bead suspension to recapture the hybrids as described above. These beads were washed three times in the same manner as the first set, the hybrids released and recaptured by a third set of beads. This set of beads was washed three times in the same manner, and additionally, three times with 0.2 ml of a solution containing 0.1M KCl, 1 mM EDTA, 10 mM Tris-HCl, pH8.0, 0.5% NP-40.

Detailed Description Text (78):
A library of template DNAs can be generated by synthesis of an oligonucleotide having in 5' to 3' order: (1) a short (for example, 40 nucleotides) sequence identical to target, (2) a short stretch (15 nucleotides) of random sequence, and (3) a sequence complementary to the 3'-most 30 nucleotides of MDV-1 RNA. The random sequence element may be generated with ease by addition of all four blocked nucleotides during synthesis of the oligonucleotide, a procedure easily accomplished with currently available automated synthesizers. A second oligonucleotide is then synthesized which is complementary to the nineteen 5'-most nucleotides of the first oligonucleotide. This is annealed to the first oligonucleotide and extended with a DNA-dependent DNA polymerase to produce a double-stranded DNA fragment. This fragment is then cleaved with the restriction enzyme Bst EII and the cleavage product ligated to a fragment of a MDV cDNA clone such as described by Lizardi (supra), previously cleaved with Bst EII. Two picomoles of the ligation product is then transcribed by T7 RNA polymerase to generate a population of RNAs containing 10.sup.12 different spacer sequences.

Detailed Description Text (81):
The sensitivity of detection of the cloned RNAs may be determined as follows. The plasmid DNAs from each clone is purified by standard methods. Each is restricted with a restriction enzyme immediately downstream of the cDNA and transcribed by T7 RNA polymerase to produce an RNA product devoid of vector sequence. This RNA is purified either by gel electrophoresis or by capture onto oligo dT-magnetic beads as in the method described in Example 2 with a DNA oligonucleotide of the same sequence as the target tailed with dA.sup.150, except that a second capture probe is omitted. Each of the purified transcripts is serially diluted in water and aliquots (10 .mu.l) added to Q.beta. reactions as described in Example 2. The reactions are allowed to run 30 min. at 37.degree. C., stopped, and the minimum number of probes required to observe product determined by the highest dilution producing fluorescence. This process is repeated for clones obtained from each round of the selection process.

Detailed Description Text (82):
It will be readily appreciated that the above process may also be applied to the isolation of RNAs which replicate efficiently when hybridized to target. This is simple performed by first hybridizing the population of RNAs to a synthetic target prior to the formation of initiation complexes. As will also be recognized, members of such a population may contain molecules which replicate more efficiently when hybridized to target than when free. Such molecules will be preferred probes for use in hybridization assays since the molecules non-specifically carried through the hybridization process will be intrinsically less efficiently replicated.

Detailed Description Text (94):
To obtain a ribozyme probe for the RNA bearing the CAUUAC (SEQ ID NO:41) element, an oligonucleotide having the sequence:
5'-TACCAGGTAATATACCACAACGTGTGTTTCTCTGGTATGATTCTCATTACGAGACAGCAGTACAAATGGCA GTATTCATCCACAATTTTCCCTATAGTGAGTCGTATTAAT-3' (SEQ ID NO:44) was synthesized. This was annealed to an oligonucleotide of the sequence 5'-ATTAATACGACTCACTATAGGG-3' (SEQ ID NO:45) ("T7 Promoter-Primer", Promega Sciences, Madison, Wis.) and transcribed with T7 RNA polymerase under conditions described by Milligan et al. (ibid), and the product RNA purified by denaturing polyacrylamide gel electrophoresis. This yielded an RNA of the sequence:
5'GGGAAAAUUGUGGAUGAAUACUGCCAUUUGUACUGCUGUCUCGUAAUGAGAAUCAUACCAGAGAAACACACG UUGUGGUAUAUUACCUGGUA-3' (SEQ ID NO:46). When annealed to HIV target nucleic acid with the MDV probe-bearing the CAUUAC (SEQ ID NO:41) inhibitory sequence element, the structure similar to that represented in FIG. 3 is obtained.

Detailed Description Text (95):
To test for the cleavage activity, 10 fmol of .sup.32 P-labelled midivariant probe and 100 fmol ribozyme probe were annealed to 1 ng of a synthetic HIV target RNA, and the hybrid complexes isolated by reversible target capture on magnetic particles, as generally described in GENE-TRAK Systems HIV assay, which assay is commercially available, except that only two rounds of capture and release were performed. After washing the final magnetic particles to which the complexes were immobilized with 0.3M KCl as described, the particles were resuspended 100 .mu.l 0.05M Tris-HCl, pH7.8, 15 mM MgCl.sub.2, and 0.5% NP-40, and incubated at 37.degree. C. for 15 minutes. The particles were removed from solution by placing in a magnetic field, and the supernatant removed and counted. Twenty seven percent of the cpm initially bound to the particles was released into solution, compared to less than one percent in a control reaction in which the ribozyme probe was omitted.

Detailed Description Text (98):
Specific placement of an affinity ligand on the portion of a cleavable midivariant probe which is distal to the cleavage directed by the release agent would allow an additional degree of discrimination of target-bound from non-hybridized probes. Briefly, the 3' terminal region of the midivariant probe such as that described in Example 10 is derivatized with biotin, poly rA or other ligand. Following hybridization with target nucleic acid, the derivatized kprobe is captured on a receptor-derivatized solid support irrespective of whether it is target-associated or not. Cleavage by the ribozyme or other release agent specifically releases only those probes which are target-associated into solution. This approach has the advantage that a high affinity ligand:receptor interaction (i.e. -biotin: avidin) may be used, and that capture is directed at the entire population of probe molecules.

Detailed Description Text (99):
As will be recognized by those familiar to the art, several methods may be used to specifically label the 3' terminal region of the probe molecule. These include, but are not limited to: (1) ligating a small RNA or DNA oligonucleotide produced synthetically, containing one or more biotin ligands, to the 3' terminus with T4 RNA ligase; (2) addition of an RNA tail to the 3' terminus with E. coli poly A polymerase, using biotinylated ribonucleoside triposphates; (3) periodate oxidation of the 3' terminal residue followed by coupling to a biotinylated ligand bearing a side chain having a primary amine followed by reduction; and, (4) hybridization of a biotinylated complementary RNA to a region distal (e.g. -3' to) the target-binding region of the midivariant probe. As will also be recognized, other ligand:receptor systems may be used, although biotin:avidin is preferred for its high affinity. These systems include, but are not limited to: (a) poly rA: poly T interactions or other hybrid-forming nucleic acids (b) specific RNA binding proteins such as the coat protein of R17, and (c) high affinity antibody:antigen interactions, such as fluorescein:antifluorescein.

Detailed Description Text (102):
A hybridization reaction containing 100 fmol of the ribozyme probe from Example 10, 10 fmol of the transcription product from the KasI-restricted DNA, 100 fmol of the biotinylated cRNA, and various amounts of synthetic HIV target are combined with poly(dA)-talled capture probes in 2.5M GTC. After 30 minutes of hybridization, the

complexes are captured onto oligo dT.sub.14 -derivatized paramagnetic particles as generally described in GENE-TRAK Systems HIV assay, which assay is commercially available. The particles are separated from the solution by placing the tubes in a magnetic field, the supernatants removed, and the particles washed three times with a buffer containing 1M GTC. The complexes are released by placing the particles in 2.75M GTC at 37.degree. C. for 5 minutes. The particles are removed, and the supernatant added to a suspension of streptavidin-derivatized paramagnetic particles (Advanced Magnetics, Cambridge, Mass.). The suspension is incubated 5 minutes at 37.degree. C., the particles separated from the solution in a magnetic field as above, washed three times with 200 .mu.l of 0.1M KCl, 1 mM EDTA, 0.5% NP-40, and 0.05M Tris-HCl pH7.8. After washing, the particles are suspended in 100 .mu.l of 15 mM MgCl.sub.2, 0.5% NP-40, 0.05M Tris-HCl, pH7.8 and incubated 15 minutes at 37.degree. C. The released midivariants are amplified by mixing 50 .mu.l of the supernatant from the above reaction with 50 .mu.l of a solution containing 2 .parallel.g of Q.beta. replicase, 800 .mu.M each ATP, GTP, CTP, and UTP, 15 mM MgCl.sub.2, and 6.4 .mu.g/ml propidium iodide. The fluorescence displayed by the reaction is followed using a Fluoroskan instrument (Flow laboratories) or other fluorimeter capable of maintaining the reaction vessel at a constant temperature of 37.degree.. The time at which a fluorescence increase is first detected is inversely proportional to the level of HIV target RNA added to the initial hybridization.

Other Reference Publication (7):

Ruffner et al., Sequence Requirements of the Hammerhead RNA Self-Cleavage Reaction, Biochemistry 29:10695-10702, 1990.

CLAIMS:

1. A composition comprising first and second nucleic acids;

said first nucleic acid comprising first, second, and third sections connected in series; wherein

said first section comprises a nucleotide sequence which is autocatalytically replicated in the presence of an RNA-dependent RNA polymerase upon cleavage of said second section;

said second section comprises a nucleotide sequence which is cleaved by a release means; and

said third section comprises a nucleotide sequence which binds to a target nucleic acid; and

said second nucleic acid comprising a first region comprising a nucleotide sequence which binds to said target nucleic acid, and a second region comprising a release means, or a portion thereof, said release means cleaving said nucleotide sequence of said second section of said first nucleic acid only when said third section of said first nucleic acid and said first region of said second nucleic acid are bound to said target nucleic acid.

4. The composition of claim 2, wherein said ribozyme is a hammerhead ribozyme.

5. The composition of claim 4, wherein said ribozyme comprises nucleotides from said target nucleic acid.

6. The composition of claim 2, wherein

(a) said second section of said first nucleic acid comprises the sequence 5'-K'UHJ-3' (SEQ ID NO:50); and

(b) said target nucleic acid and said portion of said ribozyme in said second region of said second nucleic acid comprise either 5'-MGAAAK-3' (SEQ ID NO: 2) or 5'-J'CUGANGAM'-3' (SEQ ID NO:3), independently, wherein H is C, U, or A, N is C, U, G, or A, and the letters J, J', K, K', M, and M' each represent a group of four or more nucleotides, wherein J and J' are complementary to each other, K and K' are complementary to each other, and M and M' are complementary to each other.

7. The composition of claim 6, wherein said target nucleic acid comprises the sequence 5'-MGAAAK-3' (SEQ ID NO:2) and said portion of said ribozyme in said second region of said second nucleic acid comprises the sequence 5'-J'CUGANGAM'-3' (SEQ ID NO:3), wherein N is C, U, G, or A.

8. The composition of claim 6, wherein said target nucleic acid comprises the sequence 5'-J'CUGANGAM'-3' (SEQ ID NO:3) and said portion of said ribozyme in said second region of said second nucleic acid comprises the sequence 5'-MGAAAK-3' (SEQ ID NO:2), wherein N is C, U, G, or A.

10. The composition of claim 1, wherein said portion of said release means is a DNA oligonucleotide which

(a) binds to said nucleotide sequence in said second section of said first nucleic acid when said third section of said first nucleic acid and said first region of said second nucleic acid are bound to said target nucleic acid; and

(b) causes cleavage of said nucleotide sequence in said second section of said first nucleic acid in the presence of ribonuclease H.

14. The composition of claim 1, wherein said third section further comprises a ligand which binds to an anti-ligand bound to a support.

20. A method for detecting the presence of a target nucleic acid in a sample, said method comprising the steps of

(a) contacting said sample with the composition of claim 1 to form a mixture;

(b) imposing hybridization conditions on said mixture to allow formation of a complex between the first and second nucleic acids in said composition and said target nucleic acid, if present;

(c) imposing release reaction conditions and autocatalytic replication reaction conditions on said mixture to form an autocatalytic reaction product; and

(d) detecting said autocatalytic reaction product as a measure of the presence of said target nucleic acid in said sample.

21. The method of claim 20, wherein said third section of said first nucleic acid further comprises a ligand which binds to an anti-ligand bound to a support;

said method further comprising the steps of binding said first nucleic acid to said support through said ligand and said anti-ligand, and separating substantially all unbound material from said support prior to imposing said release reaction conditions and said autocatalytic replication reaction conditions on said mixture.

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INVENTOR-INFORMATION:

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US-CL-CURRENT: 800/8; 514/44, 800/13

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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TITLE: Catalytic nucleic acid and methods of use

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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US-PAT-NO: 6365730
DOCUMENT-IDENTIFIER: US 6365730 B1

TITLE: DNA-Armed ribozymes and minizymes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 4. Document ID: US 6306607 B1

L10: Entry 4 of 14 File: USPT Oct 23, 2001

US-PAT-NO: 6306607
DOCUMENT-IDENTIFIER: US 6306607 B1

TITLE: Heterogeneous assay for pyrophosphate

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 5. Document ID: US 6238917 B1

L10: Entry 5 of 14 File: USPT May 29, 2001

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TITLE: Asymmetric hammerhead ribozymes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 6. Document ID: US 6232075 B1

L10: Entry 6 of 14 File: USPT May 15, 2001

US-PAT-NO: 6232075
DOCUMENT-IDENTIFIER: US 6232075 B1

TITLE: Heterogeneous assay for pyrophosphate detection

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 7. Document ID: US 6214546 B1

L10: Entry 7 of 14 File: USPT Apr 10, 2001

US-PAT-NO: 6214546
DOCUMENT-IDENTIFIER: US 6214546 B1

TITLE: Detection of biomolecules

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 8. Document ID: US 6083744 A

L10: Entry 8 of 14 File: USPT Jul 4, 2000

US-PAT-NO: 6083744
DOCUMENT-IDENTIFIER: US 6083744 A

TITLE: DNA-armed ribozymes and minizymes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 9. Document ID: US 6080851 A

L10: Entry 9 of 14 File: USPT Jun 27, 2000

US-PAT-NO: 6080851
DOCUMENT-IDENTIFIER: US 6080851 A

TITLE: Ribozymes with linked anchor sequences

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 10. Document ID: US 6037137 A

L10: Entry 10 of 14 File: USPT Mar 14, 2000

US-PAT-NO: 6037137
DOCUMENT-IDENTIFIER: US 6037137 A

TITLE: Fluorogenic peptides for the detection of protease activity

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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Term	Documents
RIBOZYME.DWPI,EPAB,JPAB,USPT,PGPB.	6961
RIBOZYMES.DWPI,EPAB,JPAB,USPT,PGPB.	6590
CHANGE.DWPI,EPAB,JPAB,USPT,PGPB.	1633685
CHANGES.DWPI,EPAB,JPAB,USPT,PGPB.	1465716
CONFORMATION$	0
CONFORMATION.DWPI,EPAB,JPAB,USPT,PGPB.	23639
CONFORMATIONA.DWPI,EPAB,JPAB,USPT,PGPB.	3
CONFORMATIONAI.DWPI,EPAB,JPAB,USPT,PGPB.	1
CONFORMATIONAL.DWPI,EPAB,JPAB,USPT,PGPB.	10693
CONFORMATIONALDEPENDENT.DWPI,EPAB,JPAB,USPT,PGPB.	1
CONFORMATIONALLBY.DWPI,EPAB,JPAB,USPT,PGPB.	1
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L11: Entry 8 of 11 File: USPT Sep 11, 2001

DOCUMENT-IDENTIFIER: US 6287765 B1
TITLE: Methods for detecting and identifying single molecules

Brief Summary Text (428):
Structure-activity space (SAS), also referred to herein as molecular diversity, means the diversity space of the set of all molecules net of the diversity space of structural shapes as defined herein. The multidimensional diversity in structure-activity-shape space of the instant polydiverse nucleotide libraries distinguishes them from prior art nucleic acid libraries diversified only in sequence and/or nucleotide chemistry, e.g., for selection of aptamers, ribozymes or chemically modified nucleotides. Enhanced ribozyme activity has been demonstrated using a contiguous allosteric deoxynucleotide sequence and by 2'-O-methylation (Goodchild (1992) Nucleic Acids Research 10:4607-4612). A ribozyme-based diagnostic method capable of detecting nonoligonucleotide analytes has also been described (Bockman et al. (1993) International Conference on Nucleic Acid Medical Applications, Cancun, Mexico, January 26-30), implying use of a ribozyme with both catalytic and specific binding properties. An allosteric molecular switch comprising internally hybridizable switch sequences and a DNA-binding biological recognition site has also been described (i.e., Lizardi et al., U.S. Pat. No. 5,118,801). However, the prior art does not provide access to the diversity space encompassed by MOLECULAR MACHINES comprising defined sequence segments, plastic segments, synthetic templates and/or molecular scaffolds of the instant invention.

Brief Summary Text (482):
In a preferred embodiment of the instant invention, template-directed assembly may be used to produce a promolecular delivery device comprising a payload molecule(s) specifically bound in inactive, quasireversible, releasable and/or activatable form to a designer receptor (as distinct from a target receptor, targeted receptor or disease target). The promolecular delivery device is capable of binding, storing, preserving, stabilizing, transporting, delivering, releasing and/or attaching the payload molecule in such manner that device binding to a selected target via a second recognition site (i.e., a targeting site) results in delivery, concentration, localization, release and/or activation of payload molecule(s) at a desired site of action (e.g., a selected molecule or selected nucleic acid sequence comprising a pollutant, contaminant, plant pathogen, biological weapon, toxic chemical, oil spill, microbe, virus, disease marker, or therapeutic receptor). The payload molecule is bound to a first molecular recognition or shape recognition site (i.e., a designer receptor) of the promolecular delivery device template in inactive and/or unavailable form. The resulting promolecule complex (i.e., payload-designer receptor complex) is analogous to a prodrug complex comprising a drug bound in inactive form to a designer receptor selected to bind and occupy the active site of the drug. The difference is that a promolecule complex may comprise as payload molecule not only a drug, but any nucleotide or nonnucleotide molecule or structural shape (e.g., a surface feature comprising a buckyball, nanotube or nanorod) which is capable of performing a useful function when delivered to a selected target. The payload is preferably a selected molecule (e.g., a ligand, structural molecule or effector molecule) or a selected nucleic acid sequence (e.g., an aptamer, ribozyme, antisense or triplex-forming nucleotide). The second, optionally allosteric, specific recognition site comprising a promolecular delivery device is responsible for delivering and/or releasing the payload molecule(s) to selected molecules or selected nucleic acid sequences comprising, attaching or neighboring a selected target.

Brief Summary Text (614):
Heteropolymeric functional coupling of the instant invention does not include the interaction between a ribozyme and its biological recognition site, i.e., the catalytic activity resulting from ribozyme-based recognition and cleavage of a biological nucleic acid sequence. Also outside the scope of the instant invention are ribozymes comprising synthetic defined sequence segments that bring the ribozyme catalytic element under allosteric control, i.e., by specific recognition of a selected molecule or selected nucleic acid sequence that regulates ribozyme catalytic activity.

L10: Entry 7 of 14 File: USPT Apr 10, 2001

DOCUMENT-IDENTIFIER: US 6214546 B1
TITLE: Detection of biomolecules

Brief Summary Text (10):
The use of ribozymes for diagnostic purposes has been only seldomly mentioned. WO 94/13833 describes a method for detecting nucleic acid molecules in a solution by tailoring a specific ribozyme molecule having two regions, one complementary to the nucleic acid sequence to be detected, and the other complementary to a co-target molecule bearing a detectable label. The ribozyme is able to specifically and reversibly bind both a selected target nucleic acid sequence and to the labelled co-target. When both the target and the co-target are bound, the ribozyme undergoes a conformational change which renders it active and able to cleave the label off the co-target, and the free label can then be detected. Upon cleavage of the co-target, the ribozyme is able to re-associate with an additional co-target, cleaving more label and producing more detectable signals.

L10: Entry 6 of 14 File: USPT May 15, 2001

DOCUMENT-IDENTIFIER: US 6232075 B1
TITLE: Heterogeneous assay for pyrophosphate detection

Other Reference Publication (5):
Araki et al., "Allosteric regulation of a ribozyme activity through ligand-induced conformational change," Nucleic Acids Res., 26:3379-3384 (1998).

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L10: Entry 5 of 14 File: USPT May 29, 2001

DOCUMENT-IDENTIFIER: US 6238917 B1
TITLE: Asymmetric hammerhead ribozymes

Detailed Description Text (10):
It is usually the case that substrates which form relatively short helices I and III with hammerhead ribozymes are cleaved more rapidly than substrates which create longer binding helices (see table 1). It may be that one or other of the binding helices was required to be relatively weak to allow rapid conformational changes in the ribozyme-substrate complex. Two independent symmetric (10+10) hammerhead ribozymes were found to cleave substrates which formed a helix I of 5 nucleotides at a rate several orders of magnitude faster than substrates which formed a helix III of 5 nucleotides, the other helix being 10 nucleotides in each case. The phenomenon is observed for both all-RNA and DNA-armed ribozymes. Similar results are obtained when the length of the helices I and III are limited by the length of the hybridizing arms of the rlbozymes; a symmetric 21-mer substrate is cleaved more than 100 fold faster by an asymmetric ribozyme with 5 hybridizing nucleotides in its 5' arm and 10 hybridizing bases in its 3' arm than the converse (10/5) asymmetric ribozyme. Preferred cleavage sites in the target RNA have the sequence "UH," preferably GUC, GUU, GUA, UUA and UUC. By way of example, suitable reaction conditions may comprise a temperature from about 4 degree(s) C. to about 60 degree(s) C., preferably from about 10 degree(s) to 45 degree(s) C., more preferably from about 20 degree(s) to 43 degree(s) C., pH from about 6.0 to about 9.0 and concentration of divalent cation (such as Mg.sup.2+) from about 0.1 to 100 mM (preferably 0.2 to 20 mM or more preferably 0.5 to 5 mM). The nucleotides of the sequences 3'-(N).sub.n NNNNNNA and NNN.sub.n (N) 5' of the compounds above may be of any number and sequence sufficient to enable hybridization with the nucleotides in the target RNA, as described herein. In addition these compounds may be covalently attached to an antisense molecule which may be 10 to 100 bases in length. Antisense sequences capable of hybridizing to an RNA in a mammal or plant are well known see (Shewmaker et al. U.S. Pat. No. 5,107,065, issued Apr. 21, 1992). As the ribozyme acts as an enzyme, showing turnover, the ratio of ribozyme to substrate may vary widely.

Detailed Description Text (143):
A number of publications demonstrate major changes in interhelical angles as measured by electrophoretic mobility and other methods in response to magnesium ion concentration (Bassi, et al. 1995; Amiri, et al. 1994; Gast, et al. 1994). Magnesium ions apparently induce the hammerhead to fold in to a conformation in which helix I subtends a small angle with helix II (Bassi, et al. 1995). Interestingly, in the presence of 2 mM Ma.sup.2+ ions and only with a relatively stable helix III of 9 bp, helices I and II were found to move closer together (Amiri, et al. 1994; Gast, et al. 1994). It appears that the active form of the ribozyme has helices I and II in relatively close proximity. The magnesium ion induced flexibility in helix I seen in these studies may be related to the observations we have made here on the requirement for a degree of instability in helix I. There is however no evidence of strand dissociation in the movement of helix I seen in the electrophoretic studies.

Detailed Description Text (154):
The fact that in vitro cleavage is observed in cases where both helices I and III are very long (Homann, et al. 1993; Lo, et al. 1992) suggests the presence of an alternate path for the ribozyme-substrate complex to achieve the active conformation. It may be possible, for example, that helix II is able to loosen

enough to allow the conformational change. In support of this hypothesis, we have observed that minizymes (ribozymes lacking helix II) with 10 nucleotides in each hybridising arm invariably cleave symmetrical 21-mer substrates more efficiently than symmetrical 13-mer substrates, moreover no improvement in cleavage rate constants is observed for these minizymes cleaving 10/5 substrates compared with 10/10 substrates (where there are 5 and 10 base pairs, respectively, in helices I of the complexes). Thus the conformational flexibility possessed by the minizymes, because of their lack of helix II, allows them to attain the active conformation without dissociation of helix I.

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Search Results - Record(s) 11 through 11 of 11 returned.

☐ 11. Document ID: JP 2002528109 W WO 200026226 A1 AU 200016013 A EP 1133513 A1

L11: Entry 11 of 11 File: DWPI Sep 3, 2002

DERWENT-ACC-NO: 2000-365558
DERWENT-WEEK: 200273

TITLE: Novel functional polynucleotides comprising an actuator domain, a receptor domain, and a bridging domain useful for generating highly specific polynucleotide sensors and as genetic control elements

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

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Term	Documents
ALLOSTERIC.DWPI,EPAB,JPAB,USPT,PGPB.	1907
ALLOSTERICS.DWPI,EPAB,JPAB,USPT,PGPB.	2
RIBOZYME.DWPI,EPAB,JPAB,USPT,PGPB.	6961
RIBOZYMES.DWPI,EPAB,JPAB,USPT,PGPB.	6590
((ALLOSTERIC SAME RIBOZYME) NOT 10).USPT,PGPB,JPAB,EPAB,DWPI.	11
(ALLOSTERIC SAME RIBOZYME NOT L10).USPT,PGPB,JPAB,EPAB,DWPI.	11

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L10: Entry 2 of 14 File: USPT May 14, 2002

DOCUMENT-IDENTIFIER: US 6387617 B1
TITLE: Catalytic nucleic acid and methods of use

Brief Summary Text (7):
The use of ribozymes for diagnostic purposes has been only seldomly mentioned. WO 94/13833 describes a method for detecting nucleic acid molecules in a solution by tailoring a specific ribozyme molecule having two regions, one complementary to the nucleic acid sequence to be detected, and the other complementary to a co-target molecule bearing a detectable label. The ribozyme is able to specifically and reversibly bind both to a selected target nucleic acid sequence and to the labeled co-target. When both the target and the co-target are bound, the ribozyme undergoes a conformational change which renders it active and able to cleave the label of the co-target, and the free label can then be detected. Upon cleavage of the co-target, the ribozyme is able to re-associate with an additional co-target, cleaving more label and producing more detectable signals.

Brief Summary Text (8):
WO 94/13791 concerns a regulatable ribozyme molecule which upon binding to a ligand alters its activity on a target RNA sequence. Again, as in WO 94/13833, binding to the target causes a conformational change in the ribozymes which renders it active. An example for such a change, is in the presence in the ribozyme of a redundant sequence which masks the core region of the ribozyme. Only upon binding of the target sequence to said redundant sequence, the core becomes unmasked, and thus active.

Brief Summary Text (11):
The above publications disclose ribozymes which become catalytically active, either due to a conformational change or due to a hybridization reaction which render them double-stranded. These types of reactions may also occur spontaneously, for example, if a ribozyme is inactive due to the presence of a redundant sequence which masks its core region, this redundant sequence may either break, or open, even in the absence of the target, and thus the ribozyme will become catalytically active even without a target. Spontaneous reversion to an active state, of course renders the ribozymes impractical for diagnostic purposes.

Brief Summary Text (19):
As pointed out above, there are known ribozymes which complex with a factor (termed also target), e.g. a protein or another nucleic acid sequence which causes the ribozymes to undergo conformational change whereby the catalytic activity of the ribozyme becomes more pronounced. However, in distinction from such prior art ribozymes, the proto-nucleozyme of the invention, a priori misses an essential component and said co-factor provides said missing component. Examples of such missing components are sequences in the core region of the nucleozyme. The co-factor may be a protein which fills a gap between two ends of nucleic acid strands of the nucleozyme or which joins such two ends; or may be a nucleic acid sequence capable of binding between two free ends of the proto-nucleozyme thus filling a gap.

Brief Summary Text (20):
Given the structural an functional features of the proto-nucleozyme, when in solution, it does not have the capability of spontaneously converting into a catalytically active form, since its activation is not dependent solely on a conformational change which may occur spontaneously but rather on completion of a

missing component. This feature is a further distinction from prior art inactive ribozymes, which have the capability to spontaneously undergo conformational change, albeit at a low rate, and exert some catalytic activity even in the absence of a co-factor. Thus, unlike prior art inactive ribozymes, such as those described above in the Background of the Invention section of this specification, when the ribozymes of the invention are used, there is substantially no background activity in the absence of the co-factor. For example, when proto-nucleozymes of the invention are used in diagnosis, there is essentially no "noise", namely, there is a very high signal-to-noise ratio and there are virtually no false-positive results. By another example, when used in therapeutics, the proto-nucleozymes of the invention, will exert a very high target specificity and only in the presence of an appropriate target, the catalytic complex (the nucleozyme) will be formed and exert its activity.

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L9: Entry 33 of 36 File: USPT Nov 18, 1997

DOCUMENT-IDENTIFIER: US 5688670 A
TITLE: Self-modifying RNA molecules and methods of making

Abstract Text (1):
The invention concerns a method for creating, identifying, and isolating ribozymes capable of binding a selected ligand and catalyzing a reaction involving the selected ligand. The method entails sequential selections for ligand binding molecules and catalytic molecules. The invention also includes novel ribozymes produced by these methods.

Brief Summary Text (3):
Both the genetic and enzymatic components of the earliest cells are thought to have been RNA molecules, because RNA is the only known macromolecule that can both encode information in a heritable form, and act as a biocatalyst (Joyce, Nature 338:217, 1989). It has been proposed that modern metabolism evolved prior to the evolution of encoded protein synthesis, and that early ribozyme-catalyzed metabolic transformations form the basis of our present protein-catalyzed metabolism (Bennet et al., Proc. Natl. Acad. Sci. USA 86:7054, 1989). This proposal requires that ribozymes should be able to catalyze a broad range of chemical transformations. However, to date, known natural ribozymes, including the group I and group II introns, RNAse P, and the hammerhead and hairpin RNAs, have been shown to catalyze only a restricted range of reactions involving the RNA sugar-phosphate backbone (Wilson and Szostak, Curr. Opin. Struct. Biol. 2:749, 1992).

Brief Summary Text (5):
The invention concerns a method for creating, identifying, and isolating catalytic RNA molecules capable of binding a ligand and catalyzing a reaction modifying the catalytic RNA (or other substrate). The method entails sequential selections for ligand binding RNA molecules and catalytic RNA molecules.

Brief Summary Text (7):
The methods of the invention entail sequential in vitro selections using pools of RNA molecules which include one or more regions of random sequence. Because catalysis of a complex reaction demands both the ability to bind a non-RNA ligand and the preferential stabilization of the transition state configuration of the reactants, the number of functional ribozymes in a pool of RNA having one or more regions of random sequence may be vanishingly small. The methods of the invention overcome this difficulty through the use of sequential selections. The method of the invention entails at least two selections steps: a binding selection step for identifying in a pool of random RNA molecules those RNA molecules which are capable of binding the selected ligand and a catalysis selection step for identifying in a pool of substrate binding RNA molecules (or sequence variants of such RNA molecules) those which are capable of catalyzing a reaction which modifies the catalytic RNA (or other substrate). After each selection step, an amplification step is performed. In this amplification step, the selected molecules are amplified using PCR. Of course, as explained more fully below, the binding selection step and the catalysis selection step may include one, two, or more rounds of selection and amplification. After each round, the pool of molecules is enriched for those having the desired binding or catalysis activity. Thus, the methods of the invention effectively entail three steps: 1) selection of RNA molecules capable of binding a chosen ligand from a pool of RNA molecules having a region of random sequence; 2) generation of a pool of RNA molecules which have a ligand binding sequence which is based on the identified

ligand binding sequence of ligand-binding RNA molecules selected in step 1 as well as a region of random sequence; and 3) selection of RNA molecules exhibiting catalytic activity which modifies the RNA molecule itself or a substrate attached to the catalytic RNA. To identify catalytic RNA molecules one must tag the active molecules so that they may be partitioned from the inactive ones. This tagging is most straightforward when the reaction catalyzed by the RNA molecule modifies the catalytic RNA molecule itself. This modification can involve the formation of a chemical bond, the breaking of a chemical bond, or both. Often the modification attaches one or more new atoms to the RNA. Other desirable modifications remove one or more atoms from the RNA. To be useful for tagging the modification must render the modified molecules distinguishable from non-modified molecules. Tagging can also be accomplished by modification of a substrate attached to the catalytic RNA molecule. If all of the molecules in the pool are attached to a substrate molecule, those RNA molecules which can catalyze a reaction modifying the attached sustrate can be partitioned from the RNA molecules which do not carry out the modification.

Brief Summary Text (9):
The selected ligand can include small molecules such as drugs, metabolites, cofactors, toxins, and transition state analogs. Possible ligands also include proteins, polysaccharides, glycoproteins, hormones, receptors, lipids, and natural or synthetic polymers. Preferably, for therapeutic applications, binding of the ligand and catalysis takes place in aqueous solution under physiological or near physiological salt conditions, temperature, and pH.

Brief Summary Text (10):
It is important to note that the ligand used to identify ligand-binding RNA molecules may be, but does not have to be, the same ligand which is used in the catalyst selection step. One may wish, for example, to isolate ligand-binding RNA molecules using a first ligand (e.g., ATP) and then isolate catalytic RNA molecules with a second ligand (e.g., ATP-.gamma.-S) which can bind to the same ligand binding region.

Brief Summary Text (11):
As mentioned above, the method of the invention entails at least two selection steps. In the first step, RNA molecules capable of binding the chosen ligand are selected from a pool of RNA molecules which include one or more regions of random sequence. In the second selection step, RNA molecules capable of catalyzing a reaction modifying the RNA (or other substrate) are chosen from a second pool of random RNA molecules whose sequence is based on the sequence of one or more ligand binding RNAs identified in the first selection step.

Brief Summary Text (12):
"Random RNAs" and "random sequence" are general terms used to describe molecules or sequences which have one or more regions of "fully random sequence" and/or one or more regions of "partially random sequence." Such molecules may also include one or more regions of "defined sequence." "Fully random sequence" is sequence in which there is a roughly equal probability of each of A, T, C, and G being present at each position in the sequence. Of course, the limitations of some of the methods used to create nucleic acid molecules make it rather difficult to create fully random sequences in which the probability of each nucleotide occurring at each position is absolutely equal. Accordingly, sequences in which the probabilities are roughly equal are considered fully random sequences. In "partially random sequences" and "partially randomized sequences," rather than there being a 25% chance of each of A, T, C, and G being present at each position, there are unequal probabilities. For example, in a partially random sequence, there may be a 70% chance of A being present at a given position and a 10% chance of each of T, C, and G being present. Further, the probabilities can be the same or different at each position within the partially randomized region. Thus, a partially random sequence may include one or more positions at which the sequence is fully random and one or more positions at which the sequence is defined. Such partially random sequences are particularly useful when one wishes to make variants of a known sequence. For example, if one knows that a particular 20 base sequence binds the selected ligand and that positions 2, 3, 4, 12, 13, and 15-20 are critical for binding, one could prepare a partially random version of the 20 base sequence in which the bases at positions 2, 3, 4, 12, 13, and 15-20 are the same as in the known ligand binding sequence and the

other positions are fully randomized. Alternatively, one could prepare a partially random sequence in which positions 2, 3, 4, 12, 13, and 15-20 are partially randomized, but with a strong bias towards the bases found at each position in the original molecule, with all of the other positions being fully randomized. This type of partially random sequence is desirable in pools of molecules from which catalytic RNAs are being selected.

Brief Summary Text (15):
In the first step, nucleic acids capable of binding the ligand are identified. Beginning with a pool of nucleic acids which include one or more regions of random sequence, the method for isolating ligand-binding molecules includes contacting the pool of nucleic acid with the substrate under conditions which are favorable for binding, partitioning nucleic acids which have bound the substrate from those which have not, dissociating bound nucleic acids and substrate, amplifying the nucleic acids (e.g., using PCR) which were previously bound, and, if desired, repeating the steps of binding, partitioning, dissociating, and amplifying any desired number of times.

Brief Summary Text (16):
Several cycles of selection (binding, partitioning, dissociating, and amplifying) are desirable because after each round the pool is more enriched for substrate binding nucleic acids. One can perform additional cycles of selection until no substantial improvement in substrate binding is observed. Of course, one can also perform far fewer cycles of selection.

Brief Summary Text (17):
In many cases, sequencing of nucleic acids isolated after one or more rounds of partitioning and amplification will reveal the presence of a number of different nucleic acids. One or more of these nucleic acids can be used in the pool of nucleic acids from which catalytic nucleic acids are isolated in the second selection of the method of the invention. Alternatively, the pool for the second phase can be composed of one or more nucleic acids having sequences based on the sequences of the nucleic acids identified in the binding selection. For example, sequencing of the nucleic acids which bind the substrate may suggest one or more regions of consensus sequence, i.e., sequences which appear to be important for binding. The pool of molecules used for selection of catalytic molecules may then include nucleic acids whose sequence is based on this consensus sequence. One may also employ a partially randomized sequence based on the consensus sequence. This may permit the isolation of improved binding domains. It can also permit alterations of the binding domain which may be desirable for improved catalysis. Of course, as discussed above, the degree of randomization of the consensus sequence is generally quite low. The consensus sequence region, randomized or not, may be interspersed with and/or flanked by additional randomized regions. Thus, the sequences of the molecules in the pool of nucleic acids molecules used in the catalysis selection step can differ from that of the molecule(s) identified in the substrate selection step as molecules capable of binding the desired substrate.

Brief Summary Text (18):
Those skilled in the art can readily identify ligand-binding consensus sequences by sequencing a number of ligand-binding RNA molecules and comparing their sequences. In some cases such sequencing and comparison will reveal the presence of a number of different classes of ligand binding sequences (aptamers). In these circumstances it may be possible to identify a core sequence which is common to most or all classes. This core sequence or variants thereof can be used as the starting point for the catalysis selection. By "variant" of a ligand binding sequence is meant a sequence created by partially randomizing a ligand binding sequence.

Brief Summary Text (19):
The size of the randomized regions employed should be adequate to provide a substrate binding site in the case of the binding selection step. Thus, the randomized region used in the initial selection preferably includes between 15 and 60 nucleotides, more preferably between 20 and 40 nucleotides. The randomized region or regions used for the catalysis selection step should be of sufficient length to provide a reasonable probability of being able to include catalytic activity.

Brief Summary Text (20):
The probability that any given RNA sequence of 30, 50, 100, or even 400 bases includes a region capable of binding a chosen substrate is very low. Similarly, the probability that a given RNA sequence which includes a region capable of binding a chosen substrate also has a region capable of catalyzing a reaction involving the chosen substrate is very low. Because of this each, of the two selection steps preferably begins with a pool of molecules which is large enough and random enough to include molecules which can bind the chosen substrate in the case of the binding selection or catalyze a reaction involving the chosen substrate in the case of the catalysis selection. Accordingly, the molecules used in each initial pool include at least one fully random sequence region. Binding sites may occur at a frequency of 10.sup.-10 to 10.sup.-15 in random sequences. Thus, pool sizes are preferably greater than 10.sup.10.

Brief Summary Text (21):
It is generally not practical to prepare a population of molecules which includes all of the possible sequences of a particular random sequence. However, even where one has a population of no more than 10.sup.15 different molecules out of 10.sup.60 potential sequences, one can isolate molecules having a desired binding or catalytic activity.

Brief Summary Text (22):
The catalysis selection step involves identifying RNAs which catalyze a reaction involving the chosen ligand. The pool of molecules used at the outset of this selection step generally is composed of molecules having one or more defined or partially randomized sequences which are designed to bind to the chosen ligand ("ligand binding region") as well as a second random sequence region, preferably fully randomized which serves as the source of potentially catalytic sequences. The ligand binding region included in the molecules in this catalysis selection pool can have a sequence which is identical to an identified ligand binding sequence identified in the binding selection phase. Alternatively the sequence of this region can be based on the consensus sequence of a number of substrate binding regions identified in the first step. The region may also be a partially randomized sequenced based on either a particular substrate binding sequence or substrate binding consensus sequence. Of course, the molecules also preferably include one or more defined sequence regions which can bind isolation or amplification primers.

Brief Summary Text (23):
In order to identify molecules having catalytic activity there must be a means for partitioning those RNA molecules which have catalyzed a reaction modifying the RNA molecule (or a substrate attached to the RNA) from those which have not. The selection can be accomplished using affinity columns which will bind modified, but not unmodified molecules. Alternatively, one can employ an antibody which recognizes the modified, but not unmodified molecules. It is also possible to chemically convert modified, but not unmodified ligand, to a compound which will bind selectively to an affinity column or other selective binding material (e.g., an antibody).

Brief Summary Text (27):
It may be desirable to increase the stringency of a selection step in order to isolate more desirable molecules. The stringency of the binding selection step can be increased by decreasing ligand concentration. The stringency of the catalysis selection step can be increased by decreasing the ligand concentration or the reaction time.

Brief Summary Text (28):
One can covalently link a molecule to be modified to RNA so that catalytic RNA molecules can be isolated by isolating the modified molecule. For example, one might wish to find RNAs capable of oxidizing compound A. This might be accomplished by isolating RNA molecules capable of binding a redox co-factor (NAD, FAD, or NADP). A pool of random RNAs is then created which are capable of binding the cofactor. Compound A is then covalently attached to the RNA molecules in this pool and a selection is carried out which isolates molecules having the oxidized form of compound A. Methods for linking various compounds to RNA are well known to those skilled in the art and include the use of a thiophosphate group and the use of

amines linked via a 5' phosphate.

Brief Summary Text (30):
In one aspect, therefore, the invention features a method for producing a catalytic RNA molecule capable of binding a first ligand and catalyzing a chemical reaction modifying the catalytic RNA molecule. The method includes the following steps:

Brief Summary Text (33):
c) isolating a first ligand-binding subpopulation of the first population of RNA molecules by partitioning RNA molecules in this first population which specifically bind the first ligand from those which do not;

Brief Summary Text (34):
d) amplifying the first ligand-binding subpopulation in vitro;

Brief Summary Text (35):
e) identifying a first ligand binding sequence;

Brief Summary Text (36):
f) preparing a second population of RNA molecules each of the RNA molecules including the first ligand binding sequence and a second region of random sequence;

Brief Summary Text (37):
g) contacting the second population of RNA molecules with a second ligand capable of binding the first ligand binding sequence; and

Brief Summary Text (46):
In another aspect, the invention features a method for producing a catalytic RNA molecule capable of binding a first ligand and catalyzing a chemical reaction modifying a first substrate molecule bound to the catalytic RNA molecule. The method entails the following steps:

Brief Summary Text (49):
c) isolating a first ligand-binding subpopulation of the first population of RNA molecules by partitioning RNA molecules in the first population of RNA molecules which specifically bind the first ligand from those which do not;

Brief Summary Text (50):
d) amplifying the first ligand binding subpopulation in vitro;

Brief Summary Text (51):
e) identifying a first ligand binding sequence;

Brief Summary Text (52):
f) preparing a second population of RNA molecules each of the RNA molecules including the first ligand binding sequence and a second region of random sequence, each of the RNA molecules being bound to the first substrate molecule;

Brief Summary Text (53):
g) contacting the second population of RNA molecules with a second ligand capable of binding the first ligand-binding sequence; and

Drawing Description Text (3):
FIG. 2 is a schematic illustration of the random RNA pool built around the ATP aptamer structure and the selection scheme (SEQ ID NO:5). The pool contained three regions of random sequence (N) for a total of 100 randomized bases. The aptamer region was mutagenized to a level of 15%. The Ban I site used to ligate the two halves of the pool is shown in gray. Constant primer binding sites are shown as thick lines. Random pool RNA was allowed to react with ATP-.gamma.-S and thiophosphorylated molecules were isolated by reaction with thiopyridine-activated thiopropyl sepharose. Non-specifically bound molecules were removed by washing under denaturing conditions. Active molecules were eluted with 2-mercaptoethanol. Constant regions: 5'-GGAACCUCUAGGUCAUUAAGA-3' (5'-end constant region) (SEQ ID NO:1); 5'-ACGUCAGAAGGAUCCAAG-3' (3'-end constant region) (SEQ ID NO:2).

Drawing Description Text (6):
FIG. 5 illustrates the sequences of molecules representing the seven major kinase classes (50 clones sequenced) (SEQ ID NO:6To SEQ ID NO:24). Arrows delimit the ATP aptamer conserved loop. The Ban I site used for pool construction (see FIG. 2) is underlined. Complementarity between the random region and the (constant) 5'-end of the RNA is shaded (Classes I and V). Both of these classes are 5'-kinases; these regions may serve to bind the 5'-end in the active site of the ribozymes. Sites of 2'-thiophosphorylation are shown as white letters in black boxes. Clone Kin. 47 is inactive, and contains a G to A mutation at the site of 2'-thiophosphorylation. The sequences of the constant primer binding regions (see FIG. 2) are not shown except for the first three bases following the 5' primer binding site (AGA). The length of the original pool (not including primer binding sites) was 138 nucleotides. Point deletions may have occurred during the chemical synthesis of the pool DNA, and larger deletions may be due to annealing of primers to sites in the random regions during reverse-transcription or PCR.

Drawing Description Text (9):
FIG. 8A is a strategy for in vitro evolution of self-alkylating ribozymes. FIG. 8B is a scheme for isolating biotin-binding RNAs by affinity chromatography. FIG. 8C is a scheme for isolating self-biotinylating RNA enzymes. FIG. 8D shows coding sequences for RNA pools used for in vitro selection experiments (SEQ ID NO:32 to SEQ ID NO: 34). Upper case A, C, G, T: pure nucleotide. N: equimolar mix of A, C, G, T. Lower case a, c, g, t: 70% major nucleotide, 10% each of three minor nucleotides. Underline: constant primer sequences used for amplification.

Drawing Description Text (10):
FIG. 9A illustrates progress of the biotin aptamer selection. Biotin-eluted RNA expressed as a percentage of total RNA applied to the biotin-agarose column is plotted as a function of selection cycle. Individual RNAs eluted from the seventh round were subcloned and sequenced. Greater than 90% of the clones correspond to the sequence shown in FIG. 12. FIG. 9B illustrates progress of the self-biotinylation selection. Ligation rate determined by incubation with 200 .mu.M BIE followed by streptavidin-agarose purification. Values are corrected for 0.02% non-specific RNA binding.

Drawing Description Text (12):
FIG. 11 illustrates functional biotin binder and biotin ligator sequences (SEQ ID NO:35 to SEQ ID NO:86). The partially-randomized pool sequence is shown above each set of sequences. Deviations from the principle nucleotide at each position are explicitly written while conservation of the wide type base is indicated with a dash. Biotin aptamer and self-biotinylating RNA partially-randomized pools were re-selected for biotin-agarose binding and self-biotinylation respectively. Biotin aptamer sequences correspond to clones from the fourth round of re-selection. Self-biotinylating ribozyme clones were sequenced after eight rounds of re-selection, when the overall biotinylation activity of the pool was 100 times the activity of the initial BL8-6 ribozyme. Arrows are used to indicate the locations of proposed helices. Boxed nucleotides are highly conserved yet not involved in secondary structure.

Detailed Description Text (3):
In one example of the invention, RNA molecules which bind ATP were first isolated from a pool of random RNA. RNA molecules capable of binding ATP were sequenced, and the information obtained was used to design a second pool of RNA molecules which included an ATP binding site or variant thereof. This pool was then subjected to selection and amplification to identify RNA molecules having polynucleotide kinase activity.

Detailed Description Text (4):
Selection of ATP-binding RNAs

Detailed Description Text (5):
The selection of ATP-binding RNAs was carried out in a manner designed to ensure selection of RNAs capable of binding ATP in solution as well as on an insoluble support. This was accomplished by selecting RNA molecules which bound an ATP-sepharose column and could be eluted using ATP.

Detailed Description Text (6):
A chemically synthesized pool of DNA molecules containing a central region of 120 random nucleotides 5 flanked by constant regions used as primer binding sites was PCR-amplified and transcribed in vitro by T7 RNA polymerase in the presence of [.alpha.-.sup.32 P]GRP. RNA was ethanol-precipitated and unincorporated nucleotides removed by Sephadex-G50 chromatography. Following a brief incubation at 65.degree. C. in binding buffer (300 mM NaCl, 20 Mm Tris, pH 7.6, 5 mM MgCl.sub.2), the RNA was cooled to room temperature before being loaded onto a 1-ml ATP agarose affinity column. The affinity matrix contained .about.1.6 mM ATP linked through its C8 position through a diaminohexyl linker to cyanogen bromide-activated agarose (Sigma, St. Louis, Mo.). After washing with 6 column-volumes of binding buffer, bound RNAs were eluted with 3 column-volumes of binding buffer containing 4 mM ATP, then concentrated by precipitation with ethanol. For the first three cycles, an agarose pre-column was used to prevent enrichment of the RNA pool with agarose-binding RNAs, and bound RNAs were eluted with 5 mM EDTA in water rather than affinity-eluted with ATP. After reverse transcription and PCR amplification, DNA templates were transcribed and the resulting RNA was used in the next round of selection. RNA from the eighth round of selection was converted to cDNA, amplified as double-stranded DNA by PCR, digested with EcoR1 and BamH1 gel-purified and cloned into the phage M13 based vector pGem3Z (Promega, Madison, Wis.).

Detailed Description Text (7):
Thirty-nine clones from the eighth cycle RNA population were sequenced seventeen different sequences were found. Of these, the most abundant sequence (C8-ATP-3) occurred 14 times, and 12 sequences occurred just once. Comparison of the seventeen different sequences revealed an 11-nucleotide consensus sequence, of which seven positions are invariant among all clones but one (C8-ATP-15). Clones 2, 3, 8, 15, and 19 were individually tested for binding to ATP-agarose. All had a dissociation constant (K.sub.d) of less than 50 .mu.M, except for C8-ATP-15, for which the estimated K.sub.d was .about.250 .mu.M.

Detailed Description Text (8):
To determine the minimal sequence for ATP binding, deletions of C8-ATP-3 were analyzed. An active RNA molecule 54 nucleotides in length (ATP-54-1) was generated by a combination of 5' and 3' deletions. This RNA can be folded into a secondary structure in which the 11-base consensus is flanked by two base-paired stems. Deletion of the left-hand stem abolished ATP-binding activity; dimethylsulphate modification experiments also supported the proposed secondary structure. Comparing sequences of all the clones showed that they all had a potential to fold into this secondary structure. This analysis also highlighted the presence of an invariant unpaired G opposite the 11-base consensus. The orientation and distance of this G and its flanking sequences relative to the consensus sequence was variable from clone to clone. The stems flanking the conserved G and the consensus were variable in length and composition, and frequently contained G-U base pairs. The simplest explanation for the observation that all of the selected clones contained a single consensus sequence embedded in a common secondary structure is that these clones contain the shortest sequences capable of binding ATP with the necessary affinity, and that all other sequences with comparable or superior affinity are longer and hence less abundant in the initial random sequence pool.

Detailed Description Text (9):
On the basis of these findings, a smaller RNA of 40 nucleotides (ATP-40-1) was designed, in which the consensus sequence was flanked by stems of six base pairs, with the right-hand stem closed by a stable loop sequence for enhanced stability. This RNA bound ATP as well as the full-length 164-nucleotide RNA C8-ATP-3 and was used for later experiments. Variant 40-oligonucleotide RNAs were also synthesized to test the importance of the highly conserved unpaired G (residue G34 in ATP-40-1) for ATP binding. Deleting this residue or changing it to an A residue eliminated binding, confirming the results of the selection experiments.

Detailed Description Text (10):
To determine which functional groups on the ATP are recognized by the ATP-binding RNA, we examined the ability of a series of ATP analogues to elute bound ATP-40-A RNA from an ATP-agarose column. Methylation of positions 1, 2, 3, or 6 on the

adenine base, or the 3' hydroxyl of the ribose sugar, abolish binding, as does removal of the 6-amino or 2'.sub.-- hydroxyl. Positions 7 and 8 on the base can be modified without effect; this is not surprising considering that selection was for binding to ATP linked to an agarose matrix through its C8 position. Adenosine, AMP, and ATP are equally efficient at eluting the RNA, suggesting that the 5' position on the ribose moiety is not recognized by the RNA.

Detailed Description Text (11):
Isocratic elution (Arnold et al., J. Chromatography 31:1, 1986) from ATP-agarose and equilibrium gel filtration (Fersht, in Enzyme Structure and Mechanism p. 186-188, Freeman, New York, 1985) was used to measure the dissociation constant for the RNA-ATP complex on the column and in solution. The K.sub.d of ATP-40-1 was .about.14 .mu.M when measured by isocratic elution from an ATP-C8-agarose column, and 6-8 .mu.M by equilibrium gel filtration. The K.sub.d for the ATP-agarose complex is an upper estimate, because the fraction of bound ATP that is accessible to the RNA is not known. The solution K.sub.d for adenosine was similar to that of ATP (5-8 .mu.M), but the K.sub.d for dATP was not measurable (>1 mM). The K.sub.d of ATP-40-1 for its ligand dropped to 2 .mu.M when the Mg.sup.+2 concentration was raised from 5 to 20 mM. Changing the base pair U18-A33 to C-G, as found in most of the clones initially selected, further decreased the K.sub.d to 0.7 .mu.M. At almost saturating concentrations of ATP (50 .mu.M), the RNA bound .about.0.7 equivalents of ATP. The RNA likely binds its ligands with a stoichiometry of unit.

Detailed Description Text (12):
Kethoxal modification (Moozod et al., J. Mol. Biol. 187:399, 1987) was used to assess the accessibility of guanosine residues to modification. G7 and G17 within the loop, and G6 (which forms the G-C base pair on the left side of the loop), all of which are strongly protected in the absence of ATP, become highly accessible to modification by this reagent in the presence of ATP. Other guanosine residues, including G8 in the large loop, the single unpaired G opposite the loop, and Gs in the stems, are highly protected in the presence or absence of ATP. These observations suggest that the motif is highly structured both in the presence and absence of ATP, but that binding induces a conformational change in the structure of the RNA.

Detailed Description Text (15):
A pool of RNA molecules for selection of catalytic RNAs was created based on a minimal ATP aptamer core sequence (FIG. 1). The ATP aptamer core was surrounded by three regions of random sequence, 40, 30, and 30 nucleotides in length as shown in FIG. 2. The ATP-binding domain itself was mutagenized such that each base had a 15% chance of being non-wild type, to allow for changes in the aptamer sequence that might be required for optimal activity. To increase the likelihood of finding active molecules, an effort was made to create a pool containing as many different molecules as possible. Because it is difficult to obtain an acceptable yield from the synthesis of a single oligonucleotide of this length (174 nucleotides), two smaller DNA templates were prepared and linked together to generate the full length DNA pool (FIG. 2) (Bartel and Szostak, Science, 261:1411, 1993). The presence of constant primer binding sites at the 5' and 3' ends of the molecules permitted amplification by PCR. Transcription of this DNA pool yielded between 5.times.10.sup.15 and 2.times.10.sup.16 different RNA molecules.

Detailed Description Text (18):
The selection protocol demanded only that an RNA molecule contain a thiophosphate in order for it to be isolated. Reactions that could have been selected for include: transfer of the .gamma.-thiophosphate from ATP-.gamma.-S to the 5'-hydroxyl of the RNA (analogous to the reaction catalyzed by T4 polynucleotide kinase), to the 3'-end of the RNA, to an internal 2'-hydroxyl, or even to a group on one of the bases. Transfer of diphosphate (or perhaps the entire triphosphate) instead of a single thiophosphate is also possible for all of these reactions. A splicing reaction, in which ATP-.gamma.-S displaces one of the first few nucleotides of the RNA in a manner analogous to the reaction catalyzed by the Group I introns, could also occur. However, cleavage of more than the first few bases of the RNA would result in a molecule lacking a 5'-primer binding site, and such a molecule would not be amplified during the PCR step of the selection. Similarly, any reaction that blocked reverse transcription would not be selected for.

Detailed Description Text (24):
Comparison of the sequences in the seven major classes of molecules reveals significant conservation of the sequence of the original ATP binding site in some of the active RNAs. FIG. 6 shows the putative structures for the ATP aptamer regions from Classes I, III, IV and V, the classes for which an aptamer-like structure can be drawn. It appears that Classes I and III have changed significantly from the original ATP binding domain, whereas Classes IV and V are only slightly different from the ATP aptamer consensus sequence described by Sassanfar and Szostak (Nature, 364:550, 1993). Either the right or left hand stems of the Class II, VI and VII aptamer regions appear to be missing, and it seems likely that these molecules have found novel modes of binding their substrates. Using run-off transcription of synthetic DNA oligonucleotides (Milligan and Uhlenbeck, Methods Enzymol. 180:51, 1989) the RNAs corresponding to the Class I, III, IV, and V aptamer regions were produced. The Class IV aptamer RNA binds weakly to C-8 linked ATP agarose (Sassanfar and Szostak, supra), consistent with a molecule having a K.sub.d for ATP in the range of 0.05-0.5 mM. The Class I, III, and IV aptamers, on the other hand, do not detectably interact with ATP agarose, consistent with K.sub.d s>0.5 mM for ATP (if they bind ATP at all). Presumably, the corresponding classes of kinases have developed novel modes of binding ATP-.gamma.-S.

Detailed Description Text (32):
Kinetic analysis of the most active clone from each of the four major classes of kinases has revealed that they all obey the standard Michaelis-Menten kinetics expected of molecules possessing saturable substrate binding sites. Rates for each clone were determined (as described herein) at 6 different ATP-.gamma.-S concentrations, ranging from 2 .mu.M-2.5 .mu.M. Values of k.sub.cat and K.sub.m are shown in Table 1, and range between 0.03 and 0.37 min.sup.-1 and between 41 and 456 .mu.M, respectively.

Detailed Description Text (33):
The k.sub.cat for Class I-IV ribozymes compares favorably with corresponding values for naturally occurring ribozymes, which range from 0.04 to 2 min.sup.-1. Comparison of k.sub.cat /K.sub.m is difficult because most natural ribozymes have oligonucleotide substrates that form base pairs with the ribozyme's substrate binding site, leading to very low K.sub.m values. A comparison between the kinase ribozymes described here and the self-cleavage reaction catalyzed by the Tetrahymena Group I intron is particularly relevant, however, because both reactions use external small molecule substrates (ATP-.gamma.-S and guanosine nucleotides, respectively) to modify themselves. Kin.25 (Class II) phosphorylates itself with a k.sub.cat of approximately 0.3 min.sup.-1 and a k.sub.cat /K.sub.m of 6.times.10.sup.3 min.sup.-1 M.sup.-1. The Tetrahymena self-splicing intron has a k.sub.cat of 0.5 min.sup.-1 and a k.sub.cat /K.sub.m of 2.5.times.10.sup.4 min.sup.-1 M.sup.-1 (Bass and Cech, Nature 308:820, 1984). Thus, from a vanishingly small sampling of sequence space, it has been possible to isolate a molecule with autocatalytic activity essentially as good as that of a ribozyme found in nature.

Detailed Description Text (34):
Class I-IV kinases show specificity for ATP-.gamma.-S as a substrate. No reaction (<0.1% ATP-.gamma.-S rate) could be detected with GTP.gamma.S, indicating that the RNAs can discriminate between similar substrates. Interestingly, as much as 30% of the cycle 13 pool RNA can use GTP-.gamma.-S as a substrate, and thus pool 13 does contain molecules with less stringent substrate specificities. The Class I-IV kinases are also able to discriminate between ATP-.gamma.-S and ATP (k.sub.obs (ATP-.gamma.-S)/k.sub.obs (ATP): Class I=55; Class II=300; Class III=150; Class IV.gtoreq.300; 100 .mu.M ATP, ATP-.gamma.-S). Since these values are significantly larger than the three to ten fold intrinsic reactivity difference between ATP-.gamma.-S and ATP (Herschlag et al., Biochemistry 30:4844, 1991), the data suggest that the thiophosphate is important for binding, catalysis or both. Furthermore, pool 13 RNA is not detectably labeled by either ATP-.alpha.-.sup.35 S or ATP-.alpha.-.sup.32 P, suggesting that 5' splicing is not a reaction that occurs in the pool (unless the .gamma.-thiophosphate is an absolute requirement for the molecules that carry out this reaction). Rate Acceleration: The uncatalyzed background reaction for the thiophosphorylation of RNA (or guanosine) by ATP-.gamma.-S was not detectable. Based on the sensitivity of these experiments, the

lower limit for the rate acceleration of the kinase ribozymes is roughly 10.sup.5 -fold. At 70.degree. C. the rate of hydrolysis of ATP in the presence of Mg.sup.2+ is .about.4.times.10.sup.- 4 min.sup.- 1 (pH 6-8). Correcting for the temperature and 55M water, this value gives a second order rate constant of approximately 1.times.10.sup.-6 min.sup.-1 M.sup.-1. ATP-.gamma.-S should hydrolyze 3-10 times faster than ATP. Taking this factor into account, the approximate rate enhancement of the present ribozymes [k.sub.cat /K.sub.m]/[k.sub.hydrolysis], would be 6.times.10.sup.3 min.sup.-1 M.sup.-1 /.about.10.sup.-5 min.sup.-1 M.sup.-1 or 10.sup.8 -10.sup.9 fold. This enhancement corresponds to an effective molarity of 10.sup.4 -10.sup.5 M for ATP in the ATP-ribozyme complex (k.sub.cat /k.sub.hydrolysis =0.3 min.sup.-1 /10.sup.-5 min.sup.1 M.sup.-1). A comparison of first-order rate constants gives a value for the rate enhancement that is independent of substrate binding. This value is approximately 10.sup.3 fold (k.sub.cat /k.sub.hydrolysis (1.degree. order)=0.3 min.sup.1 /.about.4.times.10.sup.-4 min.sup.-1). This analysis assumes that the mechanism of hydrolysis of ATP-.gamma.-S (dissociative) is the same as that used by the kinase ribozymes.

Detailed Description Text (36):
At least one of the selected kinases is capable of catalyzing the phosphorylation of a separate RNA substrate. In particular, Kin.46 (Class I) was demonstrated to transfer the .gamma.-thiophosphate from ATP-.gamma.-S to the 5'-end of a 6-mer oligoribonucleotide with the same sequence as the 5'-end of the ribozyme. To carry out this experiment, RNA was incubated as described in FIG. 2 except that 2.5 mM ATP-.gamma.-S was used, and 100 .mu.M 5'-HO-GGAACC-3' RNA was added. The 6-mer was synthesized by run-off transcription (Milligan et al., Meth. Enzymol. 180:51, 1989) and was dephosphorylated with calf intestinal alkaline phosphatase prior to ion-exchange HPLC purification. The thiophosphorylated 6-mer marker was made by end-labelling 5'-GGAACC-3' with ATP-.gamma.-.sup.35 S using T4 polynucleotide kinase. Products were analyzed on 20% acrylamide/8M urea gels. Full-length Kin.46 was found to catalyze the reaction approximately 500-fold more slowly than the autocatalytic reaction. Part of the reason for the decreased activity is likely to be competition for the active site between the 5'-end of the RNA and the exogenous 6-mer substrate. When the 5'-constant region of the RNA is removed (via PCR with an internal 5'-primer, followed by transcription), the activity increases .about.100-fold, but is still 6 fold below that of the auto-thiophosphorylation reaction. (At saturating concentrations of 6-mer (100 .mu.M) and ATP-.gamma.-S (2.5 mM) the initial rate of thiophosphorylation is 0.05 .mu.M/min with 1 .mu.M ribozyme. In comparison, the rate of auto-thiophosphorylation for full length Kin.46 RNA (1 .mu.M) with 2.5 mM ATP-.gamma.-S is 0.3 .mu.M/min.) At 25.degree. C. the ribozyme performs approximately 60 turnovers in 24 hours, and is thus acting as a true enzyme. The cause of the lower trans activity relative to the autocatalytic activity remains unknown, but could involve slow substrate binding or improper folding of the shortened ribozyme. The off rate of the 6-mer is not limiting because no burst phase is observed in a time course of the reaction.

Detailed Description Text (37):
The identification of autocatalytic ribozymes capable of carrying out catalysis in trans, i.e., catalyzing a reaction involving the ligand and a molecule other than the ribozymes itself can be found by testing the ability of the ribozyme to act on a molecule having a sequence similar to the region of the ribozyme which is modified.

Detailed Description Text (40):
In a second example of the invention, RNAs which bind biotin were first created, identified, and isolated using a randomized RNA pool. The selected RNAs were used to prepare a second pool of partially randomized RNAs. This pool was then subjected to selection and amplification to identify RNAs capable of ligating biotin. The overall scheme is illustrated in FIGS. 8A, 8B, and 8C.

Detailed Description Text (41):
Selection of biotin-binding RNAs

Detailed Description Text (42):
A pool of approximately 5.times.10.sup.14 different random sequence RNAs was generated by in vitro transcription of a DNA template containing a central

72-nucleotide random sequence region, flanked at both ends by 20-nucleotide constant regions. This pool (random N72 pool) had the following sequence: GGAACACTATCCGACTGGCA(N).sub.72 CCTTGGTCATTAGGATCG (SEQ ID NO:3) (FIG. 8D, also SEQ ID NO:32). On average, any given 28 nucleotide sequence has a 50% probability of being represented in a pool of this complexity. The initial pool of RNA (approximately 80 .mu.g; on average, 2-3 copies of each sequence) was resuspended in a binding buffer containing 100 mM KCl, 5 mM MgCl.sub.2, and 10 mM Na-HEPES, pH 7.4, conditions chosen to favor RNA folding and to mimic physiological environments while minimizing non-specific aggregation. The solution was applied to an agarose column derivatized with 2-6 mM biotin (Sigma, St. Louis, Mo.) and subsequently washed with 15 column volumes of binding buffer. Specifically-bound RNAs were then eluted by washing the column with binding buffer containing 5 mM biotin. Ten .mu.g of glycogen and 0.3M NaCl were then added to the eluted material, and the RNA was amplified as follows. Briefly, the mixture was precipitated with 2.5 volumes of ethanol at -78.degree. C. After resuspending the selected RNA, the reverse transcriptase primer (2.5 .mu.M) was annealed at 65.degree. C. for 3 min., and reverse transcription (RT) was carried out at 42.degree. C. for 45 min. (using Superscript RT enzyme, Life Technologies, Inc.). PCR amplification was performed by diluting one-fifth of the RT reaction with the appropriate dNTPs, PCR buffer, USB Taq polymerase (United States Biochemical, Cleveland, Ohio), and 0.5 .mu.M (+) primer containing the T7 RNA polymerase promoter. A strong band of the correct size was typically observed after 8-15 cycles amplification (94.degree. C., 1 minute; 55.degree. C., 45 seconds; 72.degree. C., 1 minute). Half of the PCR reaction was used for in vitro transcription with T7 RNA polymerase (37.degree. C., overnight). The resulting RNA was purified by electrophoresis on an 8% polyacrylamide gel.

Detailed Description Text (44):
Previous RNA selections for binding to small ligands, including various dyes, amino acids, cofactors, and nucleotides, have suggested that aptamers exist at a frequency of 10.sup.-10 to 10.sup.-11 in random sequence pools. All of these ligands, however, have contained aromatic rings which could intercalate between RNA bases and/or charged groups which might interact electrostatically with the RNA backbone. The lower frequency of biotin bindings (10.sup.-15) shows that ligands lacking such groups may require a more complex binding site.

Detailed Description Text (46):
The sequence of the biotin aptamer was used to direct the synthesis of a second pool of RNAs which was screened for the presence of biotin-utilizing ribozymes (FIG. 8A). This pool contained a core of 93 nucleotides (71 nucleotides derived from the original random region plus its 22 nucleotide 5' constant region; FIG. 8D) with the wild-type nucleotide (i.e., that which was found in the original biotin aptamer incorporated at each position in the template with 70% probability (the three non-native nucleotides each occurring with 10% probability). Deletion analysis indicated that the 3' primer was not required for binding and the same sequence was therefore used for the 3' primer of the partially-randomized pool. To allow for the possibility that the 5' primer formed part of the aptamer core, the original 5' primer sequence was included in the partially-randomized region of the new pool and a different 5' primer was appended for amplification. Because of differences in the relative rates of phosphoramidite incorporation during DNA synthesis, a biased mix of all four nucleotides was prepared with molor ratios of 3:3:2:2 (A:C:G:T). This mix was added to pure phosphoramidite stocks (A and C: 64% pure stock, 36% random mix; G and T: 55% pure stock, 45% random mix) to yield mixed stocks for pool synthesis.

Detailed Description Text (49):
In particular, reaction with BIE was terminated by the addition of 100 mM .beta.-mercaptoethanol, 5 mM EDTA, 0.3M NaCl, 50 .mu.g tRNA (E. coli, RNAse-free, Boehringer-Mannheim, Indianapolis, Ind.). After five minutes, the mixture was precipitated with 2.5 volumes ethanol on dry ice. After washing and resuspension, the RNA was applied to 0.5 ml of a 50% slurry of streptavidin agarose in wash buffer (1M NaCl, 10 mM NaHepes, pH 7.4, 5 mM EDTA) that had been washed with 50 .mu.g tRNA. After rocking 30 minutes to allow streptavidin-biotin binding, the mixture was transferred to a 10 ml-column and washed with 4.times.10 ml wash buffer and 2.times.10 ml distilled water.

Detailed Description Text (50):
RNA bound to streptavidin could be affinity eluted by first saturating the free biotin-binding sites with excess biotin and then heating in the presence of 10 mM biotin at 94.degree. C. for 8 minutes. Amplification of the resultant molecules (by reverse transcription, PCR, and transcription) yielded a pool enriched for catalysts.

Detailed Description Text (51):
After three rounds of selection, an increase in the proportion of RNAs binding to the streptavidin was observed (FIG. 9B). By the fifth round, 10% of the RNA ligated the biotin substrate. To select for the most active catalysts, the incubation time was progressively shortened from 15 hours to 30 minutes to 1 minute. After eight rounds of selection, no further increase in activity was observed suggesting that the complexity of the starting pool had been exhausted. Sequencing individual clones from the selected pool showed that 50% of the ribozymes were very closely related and were derived from a single progenitor. One of these clones, BL8-6, catalyzes self-biotinylation at a rate of 0.001 min.sup.-1 in the presence of 200 .mu.M BIE.

Detailed Description Text (52):
The rate of self-biotinylation was determined by a time course experiment. .sup.32 P-labelled RNA was first resuspended in incubation buffer (100 mM KCl, 10 mM Na-Hepes, pH 7.4, 5 mM MgCl.sub.2) and allowed to equilibrate for 10 minutes at room temperature. 200 .mu.M BIE was added to the mixture and aliquots were subsequently removed after 0 to 120 minutes of incubation. Samples were quenched and affinity purified as described in Haugland, Molecular Probes Handbook of Fluoprescent Probes and Research Chemicals. Aliquots were counted in a scintillation counter following ethanol precipitation (total RNA count) and following binding to streptavidin agarose (product RNA count); the ratio of these two counts is the fraction reacted. Optimizing enzymatic activity: It seemed likely that the original RNA pool from which the BL8-6 ribozyme was derived might not saturate the space of biotin-ligating ribozymes. To test the possibility that appropriate additional mutations to the BL8-6 sequence might increase its catalytic activity, a third RNA pool was generated based on its sequence but with non-wild-type nucleotides substituted at each position with 30% probability (FIG. 8D) (using methods described above). The selection for catalytic activity was repeated as described above, but with both the reaction incubation time and the BIE concentration progressively lowered to select for the most active enzymes. After eight rounds of selection (ending with a 1 minute incubation period at 10 .mu.M BIE), active clones from the pool were sequenced and assayed for catalytic activity. Ribozymes in this pool were uniformly more active than their BL8-6 progenitor, with one clone (BL2.8-7) catalyzing self-biotinylation at a rate of 0.05 min.sup.-1 in the presence of 100 .mu.M BIE (one hundred fold more active than BL8-6).

Detailed Description Text (57):
The background rate of guanosine alkylation by BIE was determined by two independent methods. First, radiolabelled random sequence RNA (from the pool used to isolate the original biotin binder) was incubated for 24 hours with or without 200 .mu.M BIE. The specstreptncrease in the fraction bound by streptavidin agarose (0.15%) after extensive washing was taken as a measure of the background reaction. Assuming an average of 28 guanosines/RNA sequence, this fraction corresponds to a non-catalyzed alkylation rate of 2.3.times.10.sup.- 6 s.sup.-1 M.sup.-1. In a similar approach, low concentrations of [.alpha.-.sup.32 P]-GTP were incubated overnight in the presence or absence of 200 .mu.M BIE and after 12 hours, affinity purified by streptavidin agarose. The fraction specifically bound (3.4.times.10.sup.-5) indicates a non-catalyzed rate of 2.3.times.10.sup.-6 s.sup.-1 M.sup.-1, in close agreement with that obtained from the RNA labelling experiment. A time course experiment with BL2.8-7 RNA yields a catalyzed biotinylation rate of approximately 8s.sup.-1 M.sup.-1. The ribozyme rate enhancement is thus approximately 3.times.10.sup.6, comparable to that of the most active catalytic antibodies although substantially less than that of many natural protein enzymes (Tramontano et al., J. Am. Chem. Soc. 110:2282, 1988; Janda et al., ibid. 112:1275, 1990). Structural differences between the biotin binder and the biotin ligator: Given that the biotin ligator arose by mutagenesis of the biotin binder sequence and that both molecules interact specifically with biotin, we expected to find significant structural similarities between the two RNAs. Simple comparison of their primary

sequences, however, failed to identify a well-conserved domain that might play a functional role; mutations appear randomly distributed along the length of the two sequences. To characterize the functional cores of the two molecules, we analyzed the sequences of active clones isolated from the two mutagenized RNA pools generated from the biotin aptamer and self-alkylating ribozyme sequences. After four rounds of reselection with the biotin aptamer-derived pool, >40% of the applied RNA bound tightly to biotin agarose. Similarly, three rounds of re-selection of the self-alkylating ribozyme-derived pool yielded a collection of RNAs with activity matching that of the original BL8-6 clone, and five additional rounds of selection increased the activity .about.100-fold. Approximately thirty individual RNAs from each of these subcloned pools were sequenced and analyzed to determine which nucleotide positions were conserved and which pairs of nucleotides covaried to maintain Watson-Crick base pairing. The results of these experiments are summarized below and in FIGS. 11, FIGS. 12A, and 12B.

Detailed Description Text (58):
Two regions of the biotin binder are very highly conserved in clones that retain binding activity (FIG. 11). Mutations at the 5' and 3' ends of the first conserved domain (changing the A.sup.53.G.sup.70 pair to either C:G or A:T) suggest a hairpin structure stabilized by a 4-base-pair Watson-Crick duplex. Seven non-paired bases in the middle of the first domain directly complement the 3'-terminal half 5 of the second conserved domain, thus suggesting a pseudoknot structure (FIG. 12A). In that the bases in these conserved domains are essentially invariant, the sequence data provide no covariational evidence for the pseudoknot. To test the proposed structure, a series of site-directed mutants was generated and assayed for binding to biotin agarose. Single-base substitutions that disrupt proposed Watson-Crick base pairs in the pseudoknot completely abolish biotin binding while compensatory second site mutations that introduce non-native Watson-Crick base pairs are able to largely restore biotin binding. These data strongly support the proposed pseudoknot model for the biotin aptamer.

Detailed Description Text (59):
Comparison of the sequences of active ribozymes from the BL8-6 re-selection indicate a striking change in structure relative to the original biotin binder. Nucleotides involved in the pseudoknot base-pairing (53-70, 101-107), virtually invariant in the biotin binders, are poorly conserved in the enzyme sequences (FIG. 11). In contrast, the ribozyme sequence in the region corresponding to the variable connecting loop of the biotin binder (nucleotides 71 to 94) appears to be well conserved, suggesting a structural role. Nucleotides that are very highly conserved in the biotin binder but not involved in the pseudoknot base pairing (. . . 5'.sup.-95 CGAAAG.sup.101 -3' . . .) are retained in the self-alkylating enzymes but with a highly conserved change to . . . 5'.sup.-95 CGUAAAG.sup.101 -3' . . . These results suggest that the change in function from biotin binding to alkylation of RNA with BIE is achieved by major structural rearrangements.

Detailed Description Text (60):
Further analysis of the BL8-6-derived sequences suggested a cloverleaf structure with several remarkable similarities to tRNA (FIG. 12B). The sequence . . . 5'-.sup.94 ACGUAAA100-3' . . . is presented as the tRNA variable stem, flanked on either side by extended duplexes (as indicated by several observed Watson-Crick covariations). The single guanosine in the variable stem serves as the internal alkylation site for the enzyme. One interpretation of these results is that the hexanucleotide segments CGAAAA and CGUAAA directly mediate the interaction with biotin in the biotin binder and the biotin ligator respectively, although they are presented in strikingly different secondary structure contexts. Comparison of ribozyme sequences from the third and eighth rounds of reselection suggest that the increase in pool alkylation activity is achieved by optimization of Watson-Crick base pairing in the cloverleaf duplexes and an increased fraction of purines (particularly adenosine) in the loop that caps helix 3.

Detailed Description Text (65):
Nucleic acids produced by the method of the invention can be used as in vitro or in vivo catalysts. In some cases the nucleic acids may be used to detect the presence of the ligand. For example, the nucleic acid may bind the ligand and catalyze a reaction which converts the ligand into a readily detectable molecule. The ribozymes

created by the method of the invention can also be used in assays to detect molecules modified by the ribozymes which are not themselves ligands, e.g., an RNA phosphorylated by a polynucleotide kinase ribozyme.

Other Reference Publication (11):
Bock et al., "Selection of single-stranded DNA molecules that bind and inhibit human thrombin", Nature 355(6):564-566, (1992).

Other Reference Publication (13):
Ellington et al., "Selection in vitro of single-stranded DNA molecules that fold into specific ligand-binding structures", Nature, 355:850-852, (1992).

Other Reference Publication (14):
Ellington et al., "In vitro selection of RNA molecules that bind specific ligands", Nature, 346:818-822, (1990).

Other Reference Publication (15):
Famulok, "Molecular Recognition of Amino Acids by RNA-Aptamers: An L-Citrulline Binding RNA Motif and Its Evolution into an L-Arginine Binder", J. Am. Chem. Soc., 116:1698-1706, (1994).

Other Reference Publication (24):
Sassanfar et al., "An RNA motif that binds ATP", Nature, 364:550-553, (1993).

CLAIMS:

1. A method for producing a self-modifying RNA molecule which binds a first ligand and carries out a chemical reaction modifying said self-modifying RNA molecule, comprising the steps of:

a) providing a first population of RNA molecules comprising RNA molecules having a first region of random sequence;

b) contacting said first population of RNA molecules with said first ligand;

c) isolating a first ligand-binding subpopulation of said first population of RNA molecules by partitioning RNA molecules in said first population which specifically bind said first ligand from those which do not;

d) amplifying said first ligand-binding subpopulation in vitro;

e) identifying a first ligand binding RNA sequence present in said amplified first ligand-binding subpopulation;

f) preparing a second population of RNA molecules comprising RNA molecules comprising said first ligand binding sequence and a second region of random sequence;

g) contacting said second population of RNA molecules with a second ligand which binds said first ligand binding sequence, said contacting occurring under conditions which allow said second ligand to participate in a chemical reaction which modifies an RNA molecule in said second population; and

h) isolating a subpopulation of said self-modifying RNA molecules from said population of RNA molecules by partitioning RNA molecules which have been modified in step g) from those which have not been modified.

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DOCUMENT-IDENTIFIER: US 5663064 A
TITLE: Ribozymes with RNA protein binding site

Abstract Text (1):
Ribozyme having a ligand binding site formed as a double-stranded RNA and a single-stranded loop, the ribozyme having enzymatic activity to cleave and/or ligate itself or a separate RNA molecule.

Brief Summary Text (5):
Six basic varieties of naturally-occurring enzymatic RNAs are known presently. Each can catalyze the hydrolysis of RNA phosphodiester bonds in trans (and thus can cleave other RNA molecules) under physiological conditions. Table I summarizes some of the characteristics of these ribozymes. In general, enzymatic nucleic acids act by first binding to a target RNA. Such binding occurs through the target binding portion of a enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through complementary base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a target RNA will destroy its ability to direct synthesis of an encoded protein. After an enzymatic nucleic acid has bound and cleaved its RNA target, it is released from that RNA to search for another target and can repeatedly bind and cleave new targets.

Brief Summary Text (6):
The specificity of the ribozyme can be altered in a predictable manner without loss of catalytic efficiency, and for this reason, such ribozymes have considerable potential for inactivating gene expression through the cleavage of targeted RNA (Cech, 1988 JAMA 260, 3030). In vivo, a targeted ribozyme may encounter many obstacles, including the ability to find its cognate substrate, fold into a catalytically active conformation, resist cellular nucleases, be able to discriminate its target among other RNAs, and eventually turn over to repeat the process.

Brief Summary Text (7):
The enzymatic nature of a ribozyme is advantageous over other technologies, such as antisense technology (where a nucleic acid molecule generally simply binds to a nucleic acid target to block its translation) since the concentration of ribozyme necessary to affect a therapeutic treatment is lower than that of an antisense oligonucleotide. This advantage reflects the ability of the ribozyme to act enzymatically. Thus, a single ribozyme molecule is able to cleave many molecules of target RNA. In addition, the ribozyme is a highly specific inhibitor, with the specificity of inhibition depending not only on the base pairing mechanism of binding to the target RNA, but also on the mechanism of target RNA cleavage. Single mismatches, or base-substitutions, near the site of cleavage can completely eliminate catalytic activity of a ribozyme (Chowrira & Burke, 1991 Biochemistry 30, 8518; Joseph et al., 1993 Genes & Develop. 7, 30). Similar mismatches in antisense molecules do not prevent their action (Woolf et al., 1992 Proc. Natl. Acad. Sci. USA, 89, 7305-7309). Thus, the specificity of action of a ribozyme is greater than that of an antisense oligonucleotide binding the same RNA site.

Brief Summary Text (8):
The following publications generally discuss ribozymes, and in particular hairpin

ribozymes. Van Tol et al., 1991 (Virology 180, 23) describe a hairpin ribozyme structure able to circularize. Hisamatsu et al., 1993 (Nucleic Acids Symp. Ser. 29, 173) describe hairpin ribozymes having a long substrate binding site in helix 1. Berzal-Herranz et al., 1993 (EMBO J. 12,2567) describe essential nucleotides in the hairpin ribozyme. Hampel and Tritz, 1989 (Biochemistry 28, 4929) describe a hairpin ribozyme derived from the minus strand of tobacco ringspot virus satellite [(-) sTRSV] RNA. Haseloff and Gerlach 1989 (Gene 82, 43) describe sequences required for self-cleavage reactions catalyzed by the (-) sTRSV RNA. Feldstein et al., 1989 (Gene 82, 53) tested various models of transcleaving motifs derived from (-) sTRSV RNAs. The hairpin ribozyme can be assembled in various combinations to catalyze a unimolecular, bimolecular or a trimolecular cleavage/ligation reaction (Berzal-Herranz et al., 1992, Genes & Develop. 6, 129; Chowrira and Burke, 1992 Nucleic Acids Res. 20, 2835; Komatsu et al., 1993 Nucleic Acids Res. 21, 185; Komatsu et al., 1994 J. Am. Chem. Soc. 116, 3692; Chowrira et al., 1994 J. Biol. Chem. 269, 25856). Increasing the length of helix 1 and helix 4 regions do not significantly affect the catalytic activity of the hairpin ribozyme (Hisamatsu et al., 1993 supra; Chowrira and Burke, 1992 supra; Anderson et al., 1994 Nucleic Acids Res. 22, 1096). For a review of various ribozyme motifs, and hairpin ribozyme in particular, see Burke, 1994 Nucleic Acids & Mol. Biol. 8, 105, eds. Eckstein and Lilley, Springer-Verlag, Germany; Ahsen and Schroeder, 1993 Bioessays 15, 299; Cech, 1992 Curr. Opi. Struc. Bio. 2, 605; and Hampel et al., 1993 Methods: A Companion to Methods in Enzymology 5, 37.

Brief Summary Text (9):
A hairpin ribozyme.circle-solid.substrate complex includes two intermolecular helices formed between the ribozyme and the target RNA (helix 1 and helix 2). The length of helix 1 can be varied substantially without affecting the catalytic activity of the ribozyme (Hisamatsu et al., 1993 supra). However, the length of helix 2 is reported to be sensitive to variation. The length of helix 2 is normally between 3 and 5 base-pairs long (Hampel & Tritz, 1989 supra; Feldstein et al. 1989 supra; Haseloff and Gerlach, 1989 supra, Hampel et al., 1990 supra; Feldstein et al., 1990 Proc. Natl. Acad. Sci. USA 87, 2623). Several reports suggest that mutations within this helix significantly inhibit ribozyme activity (Hampel et al., 1990 supra; Feldstein et al., 1990 supra; Chowrira & Burke, 1991 Biochemistry 30, 8518; Joseph et al., 1993 Genes & Develop. 7, 130). It is also believed in the art that the length of helix 2 should be between 3 and 5 bp (Hampel et al., 1988 EPO 360 257; Hampel et al., 1993 supra, Cech, 1992 supra; von Ahsen and Schroeder, 1993 supra; Hisamatsu et al., 1993 supra, Anderson et al., 1994 supra).

Brief Summary Text (12):
Jennings et al., U.S. Pat. No. 5,298,612 describe potential hammerhead ribozyme.circle-solid.protein interactions. It states:

Brief Summary Text (13):
Applicants have found that base-pairing in the group P is not required for cleavage of a target RNA. Accordingly, when nucleotide sequences X and Y are comprised solely of ribonucleotides and the group P is comprised solely of ribonucleotides, the ribonucleotides of group P may be base-paired for purpose other than to effect cleavage of a target RNA. Such purposes would include to allow the binding of cellular factors, such as RNA binding proteins or other cellular factors. Similarly, where the nucleotide sequences X and Y are comprised solely of deoxyribonucleotides, and the group P is comprised solely of deoxyribonucleotides, the deoxyribonucleotides of the group P may be base-paired for purposes other than involvement in endonuclease cleavage, such as interaction with DNA binding proteins or other cellular factors, which may, for example, effect cellular distribution of the endonuclease.

Brief Summary Text (15):
This invention concerns ribozymes having a double-stranded RNA and a single-stranded loop or single-stranded RNA-protein binding site incorporated into their structure. Binding of ligands (e.g., proteins) to these binding-sites preferably does not significantly affect the catalytic activity of the ribozyme, and may in fact improve the activity of the ribozyme. The site may be incorporated in a manner which does not significantly decrease the catalytic activity of the ribozyme. This site allows a protein to specifically bind to the ribozyme, and thereby potentially enhance

activity and create a ribonucleoprotein that may be more efficiently targeted to a RNA substrate molecule.

Brief Summary Text (17):
Specifically, in one example, we show that the introduction of an R17 coat protein binding site in a hairpin ribozyme structure does not interfere with the enzyme activity, but actually improves its cleavage rate by 2-fold. The R17 coat-protein (R17cp) originates from the E. coli R17 RNA bacteriophage. It tightly binds to a 23 nucleotides hairpin RNA (R17bs), and functions both as a translational repressor and in the initiation of phage assembly (Witherell et al., 1991 Prog. Nuc. Acid. Res. Mol. Biol. 40, 185). We show that the increase in catalytic efficiency correlates with stabilization of the ribozyme tertiary structure. The R17 coat protein binds the modified ribozyme as efficiently as its native binding site, does not reduce catalysis, and remains associated with the ribozyme during catalysis. Therefore, a ribozyme can function efficiently in vitro as a ribonucleoprotein.

Brief Summary Text (18):
This invention thus features improved ribozymes, based on the hairpin motif described by Hampel et al., in Hampel and Tritz 28 Biochemistry 4929, 1989; Hampel et al., 18 Nucleic Acid Res. 299, 1990 and Hampel et al., EP 0360257, and on other ribozyme motifs (as above), such as the hammerhead motif.

Brief Summary Text (19):
The invention provides a method for producing a class of enzymatic cleaving agents which exhibit a high degree of specificity for the RNA of a desired target. The enzymatic nucleic acid molecule is preferably targeted to a highly conserved sequence region of a target-encoding mRNA such that specific treatment of a disease or condition can be provided with either one or several enzymatic nucleic acids. Such enzymatic nucleic acid molecules can be delivered exogenously to specific cells as required. Alternatively, the ribozymes can be expressed from DNA or RNA vectors that are delivered to specific cells.

Brief Summary Text (20):
By "vectors" is meant any nucleic acid- and/or viral-based technique used to deliver a desired nucleic acid.

Brief Summary Text (21):
The site-specific binding of a ligand can affect ribozyme catalysis in vitro, and can overcome some in vivo targeting obstacles. This is shown below in a model ribonucleoprotein system using the R17 E. coli phage coat protein and the hairpin ribozyme. Those in the art will recognize that this is a non-limiting example of the invention and that other RNA binding proteins can be associated with the desired ribozymes and cause targeting to the desired site in an animal or plant, and/or increase ribozyme activity. The RNA binding protein may be naturally occurring or may be introduced in vitro or in vivo to have the desired function.

Brief Summary Text (22):
The engineered ribonucleoproteins may be used to localize a ribozyme in vivo. Here we show that extending helix 4 of a hairpin ribozyme to bind a protein improves by two fold the rate of catalysis, A UV cross-linking assay indicates that the 2-fold enhancement correlates directly with a stabilization of the tertiary structure of the ribozyme's large internal loop (loop B). We show that protein binding does not alter k.sub.cat or K.sub.M of the reaction, and that the protein remains bound during catalysis. These studies indicate that ribozymes can be engineered to efficiently function as a ribonucleoprotein in vitro.

Brief Summary Text (23):
Thus in a first aspect, the invention features a ribozyme having an RNA-binding protein-binding site. For example, the protein binding site is a double-stranded RNA sequence and a loop. The protein binding site may be provided at helix 4 of a hairpin ribozyme, or in stem II of a hammerhead ribozyme, or stem IV of HDV ribozyme or at the 5' end or the 3' end of such ribozymes (see FIG. 5). It can also be provided in non essential or non-critical loops or helixes or termini of other ribozyme motifs.

Brief Summary Text (25):
A number of ligands (e.g., proteins) have been identified that bind to specific RNA structural motifs (reviewed by Witherell et al., 1991 Prog. Nucleic. Acids Res. Mol. Biol. 40, 185). In non-limiting examples, FIGS. 12 through 17 show examples of RNA structural motifs that bind to specific ligands like proteins. These ligand-RNA interactions modulate a wide range of cellular processes like for example protein synthesis, viral packaging, RNA transport. Any RNA structural motif that specifically binds to a ligand can potentially be incorporated into the ribozyme structure, provided the catalytic activity of the ribozyme is not affected.

Brief Summary Text (26):
In a related aspect the invention features ribozymes having an associated RNA binding protein which is not naturally attached to the ribozyme, but is associated by bonding in a protein-nucleic acid interaction manner with a sequence on the ribozyme.

Brief Summary Text (27):
In another aspect the invention features a method for increasing activity of a ribozyme by increasing the length of a helix not necessary for complete ribozyme activity (e.g., helix 4 or a hairpin, Stem IV of HDV and stem II of a hammerhead) shown in FIGS. 1-5, and in particular, by increasing that length with a double-stranded RNA sequence to which a protein is able to bind, and to which a protein may be bound.

Brief Summary Text (28):
In addition, the method involves localizing a ribozyme by providing a protein binding sequence to which a localizing protein can be bound (for a review see Wilhelm and Dale, 1993 J. Cell. Biol. 123, 269).

Brief Summary Text (29):
In the most preferred embodiments, the protein binding sites shown in the figures herein are incorporated at least in part into a hairpin ribozyme motif of a structure shown in FIG. 18. Such introduction will allow binding to that ribozyme and either an enhanced or lowered enzymatic activity. Ligand binding to the ribozyme may also allow targeting of the ribozyme to various cellular sites or components.

Drawing Description Text (3):
FIG. 1 is a diagrammatic representation of the hammerhead ribozyme domain (SEQ ID Nos. 1 and 2) known in the art. Stem II can be .gtoreq.2 base-pair long, or can even lack base pairs and consist of a loop region.

Drawing Description Text (4):
FIG. 2 A-C shows the secondary structure of a hairpin ribozyme (SEQ ID No. 3), the R 17 coat protein binding site (SEQ ID No. 4), and a hairpin ribozyme with the coat protein binding site (SEQ ID No. 5) respectively. Helix 2 and helix 3 may or may not be linked by one or more nucleotides (e.g., 5 Cytidines). 3'P refers to 3' cleavage product. Arrow indicates the cleavage/ligation site.

Drawing Description Text (5):
FIG. 3 is a diagrammatic representation of the general structure of a hairpin ribozyme (SEQ ID Nos. 6 and 7). Helix 2 (H2) is provided with a least 4 base pairs (i.e., n is 1, 2, 3 or 4) and helix 5 is optional and may be provided of length 2 or more bases (preferably 3-20 bases, i.e., m is from 1-20 or more). Helix 2 and helix 5 may be covalently linked by one or more bases (i.e., r is .gtoreq.1 base). Helix 1, 4 or 5 may also be extended by 2 or more base pairs (e.g., 4-20 base pairs) to stabilize the ribozyme structure, and preferably is a protein binding site. In each instance, each N and N' independently is any normal or modified base and each dash represents a potential base-pairing interaction. These nucleotides may be modified at the sugar, base or phosphate. Complete base-pairing is not required in the helices, but is preferred. Helix 1 and 4 can be of any size (i.e., o and p is each independently from 0 to any number, e.g., 20) as long as some base-pairing is maintained. Essential bases are shown as specific bases in the structure, but those in the art will recognize that one or more may be modified chemically (abasic, base, sugar and/or phosphate modifications) or replaced with another base without significant effect. Helix 4 can be formed from two separate molecules, i.e., without

a connecting loop. The connecting loop when present may be a ribonucleotide with or without modifications to its base, sugar or phosphate. "q" is .gtoreq.2 bases. The connecting loop can also be replaced with a non-nucleotide linker molecule. H, refers to bases A, U or C. Y refers to pyrimidine bases. " " refers to a covalent bond.

Drawing Description Text (11):
FIG. 8 Effect of R17 binding site on UV cross-linking by the hairpin ribozyme.

Drawing Description Text (12):
FIG. 9 shows binding of R17 coat protein to R17 binding site (R17bs), HpR17, Hp and R17bs containing a deletion of A8 (R17.DELTA.A8).

Drawing Description Text (15):
FIGS. 12 shows non-limiting examples of RNA structural motifs that bind to a bactriophage coat proteins. R17 (SEQ ID No. 10), fr (SEQ ID No. 11), GA (SEQ ID No. 12) and Qb (SEQ ID No. 13) are examples of RNA bactriophages (Witherell et al., supra).

Drawing Description Text (16):
FIG. 13 shows non-limiting examples of RNA structural motifs that bind cellular proteins (Witherell et al., supra). L1 (SEQ ID No. 14), L4 (SEQ ID No. 18) and L10 (SEQ ID No. 21) are ribosomal protein binding domains. The fat (SEQ ID No. 15) is the trans-activator protein encoded by HIV. 70K (SEQ ID No. 16) and p57 (SEQ ID No. 20) are mammalian RNA binding proteins. IREP (SEQ ID No. 19) is the iron responsive element binding protein. The motif of .alpha.-Sarcin (SEQ ID No. 17) is also shown.

Drawing Description Text (17):
FIG. 14 shows RNA binding elements (SEQ ID Nos. 22-25) that are recognized by HIV regulatory proteins (rev and tat). Minimal rev binding elements are as described by Bartel et al., 1991 (Cell 67, 1-20).

Drawing Description Text (18):
FIG. 15 shows the general structure of RNA motif (SEQ ID No. 26) that binds theophyline with high affinity (Jenison et al., 1994 Science 263, 1425). Theophylline is used to treat people suffering from asthma, bronchitis and emphysema.

Drawing Description Text (19):
FIG. 16 shows general structures of RNA motifs (SEQ ID Nos. 27 and 28) that binds thrombin with high affinity (Kubik et al., 1994 Nucleic Acids Res. 22, 2619).

Drawing Description Text (20):
FIG. 17 shows general structures of RNA motifs (SEQ ID Nos. 29-35) that binds vascular endothelial growth factor with high affinity (Jellinek et al., 1994 Biochemistry 33, 10450). R, purines; Y, pyrimidines; K, guanosine or uridine; M, adenosine or cytidine; S, guanosine or cytidine; D, adenosine, guanosine or uridine; H, adenosine, uridine or cytidine; V, guanosine, adenosine or cytidine; N, any base.

Drawing Description Text (21):
FIG. 18 shows various positions where ligand-binding motifs or domains can be inserted in a hairpin ribozyme (SEQ ID Nos. 6 and 7). Arrows indicate the sites of insertions. Similarly, ligand-binding domains can be readily inserted at various positions in other ribozymes, provided the catalytic activity of the ribozyme is not significantly effected.

Detailed Description Text (2):
Synthesis of nucleic acids greater than 100 nucleotides in length is difficult using automated methods, and the therapeutic cost of such molecules is prohibitive. In this invention, small enzymatic nucleic acid motifs (e.g., of the hammerhead or the hairpin structure) are used for exogenous delivery. The simple structure of these molecules increases the ability of the enzymatic nucleic acid to invade targeted regions of the mRNA structure. However, these catalytic RNA molecules can also be expressed within cells from eukaryotic promoters (e.g., Scanlon et al., 1991, Proc.

Natl. Acad. Sci. USA, 88, 10591-5; Kashani-Sabet et al., 1992 Antisense Res. Dev., 2, 3-15; Dropulic et al., 1992 J. Virol, 66, 1432-41; Weerasinghe et al., 1991 J. Virol, 65, 5531-4; Ojwang et al., 1992 Proc. Natl. Acad. Sci. USA 89, 10802-6; Chen et al., 1992 Nucleic Acids Res., 20, 4581-9; Sarver et al., 1990 Science 247, 1222-1225). Those skilled in the art realize that any ribozyme can be expressed in eucaryotic cells from the appropriate DNA vector. The activity of such ribozymes can be augmented by their release from the primary transcript by a second ribozyme (Draper et al., PCT WO 93/23569, and Sullivan et al., PCT WO 94/02595, both hereby incorporated in their totality by reference herein; Ohkawa et al., 1992 Nucleic Acids Symp. Ser., 27, 15-6; Taira et al., 1991, Nucleic Acids Res., 19, 5125-30; Ventura et al., 1993 Nucleic Acids Res., 21, 3249-55;Chowrira et al., 1994 J. Biol Chem. 269, 25856).

Detailed Description Text (3):
The following are examples of a hairpin ribozyme with a protein binding sequence provided in helix 4 and methods for its production and introduction into a cell. Those skilled in the art will recognize that this example is not limiting the invention and that other protein binding sequences can be readily provided in other locations (or in other ribozyme motifs) as noted above for single-stranded or double-stranded RNA binding proteins. If they are added at the 5' or 3' end of the ribozyme they can be provided as a hairpin loop to form the double-stranded RNA.

Detailed Description Text (13):
Filter-Binding Assays

Detailed Description Text (14):
Association constants were determined by filter-binding assays (Carey et al., 1983 Biochemistry 22, 2601). Trace amount of labeled RNA (15-32 fmoles) was heat denatured and renatured in 500 .mu.l of T.M.S buffer (40 mM Tris-HCl pH 8, 12 mM MgCl.sub.2 and 2 mM spermidine) or TMK buffer (40 mM Tris-Acetate pH 8, 10 mM Mg.Acetate, 80 mM KCl). The reaction was initiated by adding R17 coat protein at various concentrations (0.01 nM to 1 .mu.M). After at least 20 minutes of incubation at the desired temperature, 450 .mu.l of the reaction mixture was filtered through pre-soaked nitrocellulose filter (Millipore-HAWP 024-0,45 .mu.M). The amount of complex retained by the filter was determined by liquid scintillation counting. The amount of input RNA was determined by spotting a 20 .mu.l aliquot of the reaction mixture on a filter. The fraction bound by the filter was plotted as a function of protein concentration. As previously described (Carey et al., supra; Schneider et al., 1992 J. Mol. Biol. 228, 862), the maximum amount of fraction complexed varied between 40 and 100% depending on the RNA preparation. Results were normalized to 100% of retention at saturation for purposes of calculations and to compare different experiments. Although a theoretical pseudo-first order binding curve does not always describe accurately the experimental data, data points were fitted to a curve describing theoretical bimolecular association (Pyle & Green, 1994 Biochemistry 33, 2716). Dissociation constant (Kd) values were then obtained from a non-linear least-square fit using sigmaplot software (Jandel Scientific). Standard error was calculated from the fit of the observed curve to the theoretical one (reported by the sigmaplot software).

Detailed Description Text (28):
Insertion of a R17 Coat Protein Binding Site into the Hairpin Ribozyme Structure

Detailed Description Text (29):
The R17 coat protein (R17cp) specifically binds a 23 nt RNA (R17bs) which forms a stable stem-loop structure (FIG. 2B; Witherell, et al., 1991 supra). Helix 4 of the hairpin ribozyme shows no sequence or length requirement (Berzal-Herranz et al., 1993 supra; Chowrira and Burke, 1992 supra; Anderson et al., 1994 supra) and the helix 4 loop can be deleted without effecting catalytic activity of the ribozyme (Chowrira & Burke, 1992 supra). Applicant has therefore introduced the R17bs in the helix 4 region of the hairpin ribozyme (FIG. 2C). The hairpin ribozyme containing the R17bs is designated HpR17.

Detailed Description Text (30):
Those skilled in the art will recognize that this example is non-limiting to the invention and that other protein binding sequences can be readily provided in other

locations (or in other ribozyme motifs) as noted above for single-stranded or double-stranded RNA binding proteins. Those skilled in the art will recognize that the protein binding domain can be inserted at any position within a ribozyme (including the termini), as long as the catalytic activity of the ribozyme is not compromised.

Detailed Description Text (32):
The Protein Binding Domain increases the Rate of RNA Cleavage Reaction Catalyzed by the Ribozyme

Detailed Description Text (35):
The Protein Binding Domain Increases the Rate of RNA Ligation Reaction Catalyzed by the Ribozyme

Detailed Description Text (38):
The Protein Binding Domain increases UV Cross-linking Efficiency

Detailed Description Text (41):
R17 Coat Protein Binds the HpR17 Ribozyme and the R17bs RNA with Similar Efficiencies

Detailed Description Text (42):
A gel-shift assay is used to determine complex formation between RNA and R17cp. R17cp binds to R17bs and HpR17 RNA efficiently. No specific protein-RNA complexes were detected with Hp and R17.DELTA.A8 (a null mutant binding site in which the bulged Adenine 8 is deleted and is used as a negative control; Carey et al., 1983 Biochemistry 22, 4723). Complex formation was observed at protein concentrations roughly equivalent to those previously reported (Lowary & Uhlenbeck, 1987 supra).

Detailed Description Text (43):
A filter binding assay (Carey, et al., 1983 Biochemistry 22, 2601) is used to confirm the results from gel-shift assay described above. Referring to FIG. 9, the R17cp binds to HpR17 as well as it binds to its genuine binding site (R17bs). In contrast, the binding curve for the Hp ribozyme is similar to the one observed with the null-mutant R17.DELTA.A8 (Carey, et al., 1983 supra). Therefore the protein does not bind specifically to the hairpin ribozyme but introduction of the binding site sequences are sufficient to enable R17cp to bind efficiently to HpR17 ribozyme. The protein was also unable to recognize and bind to the substrate RNA alone.

Detailed Description Text (44):
The of R17cp binding was assayed in the presence of standard ribozyme reaction buffer (TMS) and in a more physiological buffer (TMK=Tris-Ac pH 7 40 mM, Mg(OAc).sub.2 12 mM, KCl 80 mM) at 37.degree. C. or 25.degree. C. The Kd values are in good agreement with previous studies (Carey, et al., 1983 supra; Lowary & Uhlenbeck, 1987 supra; Schneider, et al., 1992 supra). As previously described, the efficiency of R17cp binding decreases with variations in temperature and ionic strength (Carey et al., 1983 supra). To prevent degradation by cellular ribonucleases that may have copurified with R17cp, applicant added ATA (aurintricarboxylic acid) in the reaction buffer. The binding properties were not affected by the presence of 0.1 mM ATA.

Detailed Description Text (46):
Binding of R17cp to HpR17 does not effect RNA Cleavage/Ligation Reactions Catalyzed by the Ribozyme

Detailed Description Text (51):
Applicant has shown that the R17cp binds to the HpR17 ribozyme and that the ribozyme functions efficiently in the presence of saturating concentration of the protein (see above). However, these results do not show that the ribonucleoprotein is actually responsible for the observed catalytic activity. To address this issue applicant first determined the protein affinity for HpR17 in the presence of a large excess of ribozyme over the substrate (under "ribozyme excess" RNA cleavage conditions). Applicant did not detect any differences in R17cp binding to the HpR17 ribozyme in the presence or absence of the substrate. Although the protein binds to the HpR17 under cleavage conditions, the possibility remains that the protein may

quickly dissociate and re-associate while the reaction is taking place. To rule out this possibility, applicant monitored the protein-ribozyme complex stability during catalysis. In these experiments, the protein-ribozyme complex was pre-formed, and the cleavage reaction was initiated by adding the substrate in presence of a large excess of R17 binding site (R17bs) RNA. Excess R17bs is added to trap R17cp that may dissociate from the complex during catalysis. Under these conditions, the HpR17-R17cp complex is stable for over an hour, during which most of the target RNA has been cleaved. The extreme stability of the complex is not surprising, as Lowary and Uhlenbeck showed that this RNA-protein complex has a half life of about 7 hours in TMK and over 24 h in low salt buffer at 0.degree. C. (Lowary & Uhlenbeck, 1987 supra).

Detailed Description Text (54):
Ribozymes engineered to cleave foreign target RNA sequences are sometimes accompanied by a loss in the catalytic efficiency of that ribozyme. The 2-fold increase in cleavage associated with the HpR17 ribozyme may significantly improve the efficiency of ribozymes engineered to cleave a foreign target, especially, if introduced in combination with base substitutions that have been previously shown to act as general catalysis enhancing mutations of the ribozyme (Berzal-Herranz, et al., 1993 supra).

Detailed Description Text (55):
The present invention shows that a ribozyme can function as a ribonucleoprotein. The R17 coat-protein is part of a class of stem-loop RNA binding proteins that are common in prokaryotes and eukaryotes, and the results obtained here can be extended to other proteins (see FIGS. 12-17). It has recently been shown that a ribozyme can recruit non-specific RNA binding proteins to improve its activity (Bertrand & Rossi, 1994 EMBO. J 13, 2904; Coetze, et al., 1994 supra; Herschlag et al., 1994 EMBO. J. 13, 2913; Tsuchihaschi et al., 1993, Science 262, 99-102).

Detailed Description Text (56):
The present invention features a ribozyme able to tightly bind to a specific RNA binding protein, which may have significant therapeutic applications; for example, it could be used to co-localize the ribozyme to its target within a cell, where RNAs are not homogeneously dispatched. It has been shown on several instances that RNAs are directed towards specific locations in the nucleus or the cytoplasm (Wilhelm & Vale, 1993 J. Cell Biol 123, 269). Sullenger and Cech (1993 Science 262, 1566) have introduced a ribozyme into a defective virus DNA sequence. The resulting chimeric transcript follows the same pathways as the viral RNA lacking the ribozyme and is therefore co-localized with target RNAs in the viral nucleocapsid. This strategy was shown to be effective in improving the ribozyme activity in vivo. An alternative way of directing the ribozyme to a desired location is to attach, to a ribozyme, a binding site for a protein known to be localized close to the targeted RNA (e.g., ribosomal proteins), or known to be involved in RNA transport (e.g., certain small nuclear ribonucleoproteins).

Detailed Description Text (57):
Applicant has shown in the present invention that a model HpR17 ribozyme can actually function as a ribonucleoprotein. Other engineered hairpin ribonucleoproteins also be readily generated. For example, the introduction of a portion of the Rev binding element (FIG. 14A) in the hairpin ribozyme could be used to co-localize the ribozyme with HIV mRNA (Cohli et al., 1994 Antisense Res. Develop. 4, 19).

Detailed Description Text (58):
Specific interactions between RNA and proteins are known to be involved in RNA stability and viral packaging. Depending on the protein used, a ribozyme (ribonucleoprotein) can be used to stabilize the RNA in the cell, and enable efficient transport of the ribozyme in an organism along with the targeted virus. The presence of the R17 binding site in a RNA sequence is sufficient to promote its encapsidation by the virus (Pickett & Peabody, 1993 Nucleic Acids Res. 21, 4621). Such a system could be used to transport copies of a ribozyme among a population of cells, like procaryotic cell population or inside a plant or an animal.

Detailed Description Text (61):

Protein binding domains described in the present invention can also be used to regulate ribozyme activity. In vitro evolution strategies (see Joyce, 1992 Scientific American 267, 90) are used to evolve for a protein binding-dependent ribozyme. A protein-dependent ribozyme is a useful tool for in vivo applications: e.g., it would allow the engineering of ribozymes specific for a cell type or a cell infected by a virus.

Detailed Description Text (62):
In vitro evolution strategies can also be used to evolve ribozymes that would be inhibited by binding of a specific protein to the designated binding site. Such protein-dependent catalytic RNA could also be studied as a model system for the demonstrated or supposed cellular protein dependent ribozymes: group I and II introns, RNase P, ribosome spliceosome (see for example Mohr et al., 1994 Nature 370, 147).

Detailed Description Text (63):
The in vitro selection (evolution) strategy is similar to approaches developed by Joyce (Beaudry and Joyce, 1992 Science 257, 635; Joyce, 1992 Supra), Szostak (Bartel and Szostak, 1993 Science 261,1411; Szostak, 1993 TIBS 17, 89) and Burke (Berzal-Herranz et al., 1992 Genes & Develop. 6, 129). Briefly, a random pool of nucleic acids is synthesized wherein, each member contains two domains: a) one domain consists of a region with degenerate (random) sequence. The known nucleotide sequence domain enables: 1) the nucleic acid to bind to its target (the region flanking the mutant nucleotide), 2) complementary DNA (cDNA) synthesis and PCR amplification of molecules selected for their desired activity (e.g., protein binding) 3) introduction of restriction endonuclease site for the purpose of cloning. The degenerate domain can be created to be completely random (each of the four nucleotides represented at every position within the random region) or the degeneracy can be partial (Beaudry and Joyce, 1992 supra). This random library of nucleic acids is incubated under conditions that ensure folding of the nucleic acids into conformations that facilitate the desired activity (e.g., protein binding). Following incubation, nucleic acids are converted into complementary DNA (if the starting pool of nucleic acids is RNA). Nucleic acids with desired trait (e.g., protein binding) can be separated from rest of the population of nucleic acids by using a variety of methods. For example, a filter-binding assay can be used to separate the fraction that binds the desired protein from those that do not. The fraction of the population that is bound by the protein (for example) is the population that is desired (active pool). A new piece of DNA (containing new oligonucleotide primer binding sites for PCR and RE sites for cloning) is introduced to the termini of the active pool (to reduce the chances of contamination from previous cycles of selection) to facilitate PCR amplification and subsequent cycles (if necessary) of selection. The final pool of nucleic acids with the desired trait is cloned in to a plasmid vector and transformed into bacterial hosts. Recombinant plasmids can then be isolated from transformed bacteria and the identity of clones can be determined using DNA sequencing techniques.

Detailed Description Text (64):
Target Sites

Detailed Description Text (65):
Targets for useful ribozymes can be determined as disclosed in Draper et al. WO 93/23569 Sullivan et al., WO 94/02595 as well as by Draper et al., "Method and reagent for treatment of arthritic conditions" U.S. Ser. No. 08/152,487, filed Nov. 12, 1993, and hereby incorporated by reference herein in totality. Rather than repeat the guidance provided in those documents here, below are provided specific examples, not limiting to those in the art. Ribozymes to such targets are designed as described in those applications and synthesized to be tested in vitro and in vivo, as also described. Such ribozymes can also be optimized and delivered as described therein.

Detailed Description Text (66):
Ribozymes are designed to anneal to various sites in the target RNA. The binding arms are complementary to the target site sequences described above. The ribozymes are chemically synthesized. The method of synthesis used follows the procedure for

normal RNA synthesis as described in Usman et al., 1987 J. Am. Chem. Soc. 109, 7845, and in Scaringe et al., 1990 Nucleic Acids Res. 18, 5433, and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The average stepwise coupling yields were >98%. Hairpin ribozymes are synthesized in two parts and annealed to reconstruct the active ribozyme (Chowrira and Burke, 1992 Nucleic Acids Res., 20, 2835). Alternately, ribozymes can also be transcriptionally synthesized using bacteriophage RNA polymerase, foe example, T7 RNA polymerase. Ribozymes are modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-flouro, 2'-o-methyl, 2'-H (for a review see Usman and Cedergren, 1992 TIBS 17, 34). Ribozymes are purified by gel electrophoresis using general methods or are purified by high pressure liquid chromatography (HPLC; See Usman et al., Synthesis, deprotection, analysis and purification of RNA and ribozymes, filed May, 18, 1994, U.S. Ser. No. 08/245,736 the totality of which is hereby incorporated herein by reference) and are resuspended in water.

Detailed Description Text (68):
Ribozymes are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells. The RNA or RNA complexes can be locally administered to relevant tissues ex vivo, or in vivo through injection, aerosol inhalation, infusion pump or stent, with or without their incorporation in biopolymers.

Detailed Description Text (71):
In a preferred embodiment of the invention, a transcription unit expressing a hairpin ribozyme that cleaves target RNA is inserted into a plasmid DNA vector or an adenovirus or adeno-associated DNA viral vector. Both viral vectors have been used to transfer genes to the lung and both vectors lead to transient gene expression (Zabner et al., 1993 Cell 75, 207; Carter, 1992 Curr. Opi. Biotech. 3, 533) and both vectors lead to transient gene expression. The adenovirus vector is delivered as recombinant adenoviral particles. DNA may be delivered alone or complexed with vehicles (as described for RNA above). The DNA, DNA/vehicle complexes, or the recombinant adenovirus particles are locally administered to the site of treatment, e.g., through the use of an injection catheter, stent or infusion pump or are directly added to cells or tissues ex vivo.

Detailed Description Text (72):
In another aspect of the invention, ribozymes that cleave target molecules are expressed from transcription units inserted into DNA, RNA, or viral vectors. Preferably, the recombinant vectors capable of expressing the ribozymes are locally delivered as described above, and transiently persist in target cells. Once expressed, the ribozymes cleave the target mRNA. The recombinant vectors are preferably DNA plasmids, adenovirus, retroviral or adeno-associated virus vectors. However, other mammalian cell vectors that direct the expression of RNA may be used for this purpose.

Detailed Description Text (73):
Thus, ribozymes of the present invention that cleave target mRNA and thereby inhibit and/or reduce target activity have many potential therapeutic uses, and there are reasonable modes of delivering the ribozymes in a number of the possible indications. Development of an effective ribozyme that inhibits specific function are described in the art.

Detailed Description Text (74):
By "inhibit" is meant that the activity or level of target RNA is reduced below that observed in the absence of the ribozyme, and preferably is below that level observed in the presence of an inactive RNA molecule able to bind to the same site on the RNA, but unable to cleave that RNA.

Detailed Description Text (75):
By "vectors" is meant any nucleic acid- and/or viral-based technique used to deliver a desired nucleic acid.

Detailed Description Text (77):
Ribozymes of this invention may be used as diagnostic tools to examine genetic drift

and mutations within diseased cells, or to detect specific RNA molecules, such as virus RNA. The close relationship between ribozyme activity and the structure of the target RNA allows the detection of mutations in any region of the molecule which alters the base-pairing and three-dimensional structure of the target RNA. By using multiple ribozymes described in this invention, one may map nucleotide changes which are important to RNA structure and function in vitro, as well as in cells and tissues. Cleavage of target RNAs with ribozymes may be used to inhibit gene expression and define the role (essentially) of specified gene products in the progression of disease. In this manner, other genetic targets may be defined as important mediators of the disease. These experiments will lead to better treatment of the disease progression by affording the possibility of combinational therapies (e.g., multiple ribozymes targeted to different genes, ribozymes coupled with known small molecule inhibitors, or intermittent treatment with combinations of ribozymes and/or other chemical or biological molecules). Other in vitro uses of ribozymes of this invention are well known in the art, and include detection of the presence of mRNA associated with a related condition. Such RNA is detected by determining the presence of a cleavage product after treatment with a ribozyme using standard methodology.

Detailed Description Text (78):
In a specific example, ribozymes which can cleave only wild-type or mutant forms of the target RNA are used for the assay. The first ribozyme is used to identify wild-type RNA present in the sample and the second ribozyme will be used to identify mutant RNA in the sample. As reaction controls, synthetic substrates of both wild-type and mutant RNA will be cleaved by both ribozymes to demonstrate the relative ribozyme efficiencies in the reactions and the absence of cleavage of the "non-targeted" RNA species. The cleavage products from the synthetic substrates will also serve to generate size markers for the analysis of wild-type and mutant RNAs in the sample population. Thus each analysis will require two ribozymes, two substrates and one unknown sample which will be combined into six reactions. The presence of cleavage products will be determined using an RNAse protection assay so that full-length and cleavage fragments of each RNA can be analyzed in one lane of a polyacrylamide gel. It is not absolutely required to quantify the results to gain insight into the expression of mutant RNAs and putative risk of the desired phenotypic changes in target cells. The expression of mRNA whose protein product is implicated in the development of the phenotype is adequate to establish risk. If probes of comparable specific activity are used for both transcripts, then a qualitative comparison of RNA levels will be adequate and will decrease the cost of the initial diagnosis. Higher mutant form to wild-type ratios will be correlated with higher risk whether RNA levels are compared qualitatively or quantitatively.

Detailed Description Text (84):
Requires a U in the target sequence immediately 5' of the cleavage site.

Detailed Description Text (85):
Binds 4-6 nucleotides at 5' side of cleavage site.

Detailed Description Text (91):
Hammerhead Ribozyme

Detailed Description Text (93):
Requires the target sequence UH immediately 5' of the cleavage site.

Detailed Description Text (94):
Binds a variable number nucleotides on both sides of the cleavage site.

Detailed Description Text (98):
Requires the target sequence GUC immediately 3' of the cleavage site.

Detailed Description Text (99):
Binds 4-6 nucleotides at 5' side of the cleavage site and a variable number to the 3' side of the cleavage site.

Detailed Description Text (103):
Cleavage of target RNAs recently demonstrated.

Detailed Description Text (105):
Binding sites and structural requirements not fully determined, although no sequences 5' of cleavage site are required.

Detailed Description Text (109):
Cleavage of target RNAs recently demonstrated.

Detailed Description Text (111):
Binding sites and structural requirements not fully determined. Only 1 known member of this class. Found in Neurospora VS RNA (FIG. 5).

Detailed Description Paragraph Table (3):
TABLE 2 Kinetics of RNA Cleavage by the Hairpin Ribozyme (Hp) and the Hairpin Ribozyme with R17 Binding Site (HpR17)
Reaction k.sub.cat /K.sub.M k.sub.cat /K.sub.M Conditions Ribozyme (min.sup.-1 .multidot. .mu.M.sup.-1 (rel.sup.c.)
T.M.S.sup.a, 37.degree. C. Hp 3.1 1 HpR17 5.6 1.8 T.M.S, 25.degree. C. Hp 2.1 0.7 HpR17 4.6 1.5 T.M.K.sup.b, 25.degree. C. Hp 1.2 0.4 HpR17 2.7 0.9
.sup.a T.M.S = 40 mM Tris.HCl, pH 7.5; 12 mM MgCl.sub.2 ; 2 mM Spermidine .sup.b T.M.K = 40 mM Tris.Acetate, pH 7.5; 10 mM Mg.Acetate; 80 mM KCl .sup.c Relative k.sub.cat /K.sub.M ; the second order rate constant is compared with the rate of Hp in T.M.S buffer at 237.degree. C.

Detailed Description Paragraph Table (4):
TABLE 3 Effect of R17 Protein Binding on RNA Cleavage Activity by the Hairpin Ribozyme Presence K.sub.cat /K.sub.M of R17cp K.sub.M k.sub.cat (min.sup.-1 .multidot. k.sub.cat /K.sub.M Ribozyme (0.5 .mu.M) (nM) (min.sup.-1) .mu.M.sup.-1) (rel.)
Hp - 62 .+-. 15 0.25 .+-. 0.02 4 1 Hp + 67 .+-. 20 0.24 .+-. 0.03 3.6 1 HpR17 - 51 .+-. 4 0.31 .+-. 0.005 6 1.7 HpR17 + 58 .+-. 10 0.32 .+-. 0.02 5.7 1.7

Other Reference Publication (15):
Chowrira et al., "In Vitro and in Vivo Comparison of Hammerhead, Hairpin, and Hepatitis Delta Virus Self-Processing Ribozyme Cassettes," J. Biol. Chem. 269:25856-25864 (1994).

Other Reference Publication (16):
Chowrira and Burke, "Binding and Cleavage of Nucleic Acids by the Hairpin Ribozyme," Biochemistry 30:8518 (1991) (from 208/154).

Other Reference Publication (35):
Jellinek et al., "Inhibitions of Receptor Binding by High-Affinity RNA Ligands to Vascular Endothelial Growth Factor," Biochemistry 33:10450-10456 (1994).

Other Reference Publication (52):
Pieken et al., "Kinetic Characterization of Ribonuclease-Resistant 2'-Modified Hammerhead Ribozymes," Science 253:314-317 (1991).

CLAIMS:

1. An enzymatic RNA molecule comprising: a protein binding site formed as a double-stranded RNA and a single-stranded loop, wherein said enzymatic RNA molecule is capable of specifically cleaving a separate RNA molecule, wherein said site is selected from a group shown in FIGS. 2 and 12-17 (SEQ. ID. Nos. 4, 10-16 and 18-28) inclusive, and wherein said enzymatic nucleic acid is in a hairpin motif.

2. The enzymatic RNA molecule of claim 1 or 4 wherein a protein is bound to said protein binding site in said enzymatic RNA molecule.

3. The enzymatic RNA molecule of claim 1 or 4 wherein the said protein binding site is adjacent to the 3' or the 5' end of said enzymatic RNA molecule.

4. An enzymatic RNA molecule comprising: a protein binding site formed as a

double-stranded RNA and a single-stranded loop, wherein said enzymatic RNA molecule is capable of specifically ligating separate RNA molecules, wherein said site is selected from a group shown in FIGS. 2 and 12-17 (SEQ ID Nos. 4, 10-16 and 18-28) inclusive, and wherein said enzymatic nucleic acid is in a hairpin motif.

5. The enzymatic RNA molecule of claim 1 or 4, wherein said protein binding site is capable of binding R17 protein.

6. The enzymatic RNA molecule of claim 1 or 4, wherein said protein binding site is capable of binding human immunodeficiency virus encoded TAT protein.

7. The enzymatic RNA molecule of claim 1 or 4, wherein said protein binding site is capable of binding human immunodeficiency virus encoded Rev protein.

8. The enzymatic RNA molecule of claim 1 or 4, wherein said protein binding site is capable of binding mammalian iron response element binding protein.

9. The enzymatic RNA molecule of claim 1 or 4, wherein said protein binding site is capable of binding mammalian vascular endothelial growth factor.

13. A method for increasing the activity of a an enzymatic RNA molecule in a hairpin motif by including a RNA protein binding site in the helix 4 region of said enzymatic RNA molecule.

14. A method for decreasing the activity of a hairpin ribozyme by including a RNA protein binding site in helix 4.

15. A method for localizing an enzymatic RNA molecule in a hairpin motif by providing a RNA protein binding sequence in said enzymatic RNA molecule and binding said site with a localizing protein.

End of Result Set

L9: Entry 36 of 36 File: USPT Dec 5, 1995

DOCUMENT-IDENTIFIER: US 5472840 A
TITLE: Nucleic acid structures with catalytic and autocatalytic replicating features and methods of use

Brief Summary Text (5):
The term "target" or "target molecule" in a diagnostic sense, refers to a molecule of interest, i.e. the molecule whose presence one wishes to know. In a therapeutic sense, the term "target" or "target molecule" refers to a molecule associated with a disease.

Brief Summary Text (6):
The term "biological binding pair" as used in the present application refers to any pair of molecules which exhibit mutual affinity or binding capacity. A biological binding pair is capable of forming a complex under binding conditions. For the purposes of the present application, the term "ligand" will refer to one molecule of the biological binding pair, and the term "antiligand" or "receptor" will refer to the opposite molecule of the biological binding pair. For example, without limitation, embodiments of the present invention have application in nucleic acid hybridization assays where the biological binding pair includes two complementary nucleic acids. One of the nucleic acids is designated the ligand and the other nucleic acid is designated the antiligand or receptor. One of the nucleic acids may also be a target molecule. The designation of ligand or antiligand is a matter of arbitrary convenience. The biological binding pair may include antigens and antibodies, drugs and drug receptor sites, and enzymes and enzyme substrates, to name a few.

Brief Summary Text (7):
The term "probe" refers to a ligand of known qualities capable of selectively binding to a target antiligand or receptor. As applied to nucleic acids, the term "probe" refers to nucleic acid having a base sequence complementary to a target nucleic acid. The probe and the target are capable of forming a probe target complex under binding conditions. The term "probe" will be used herein, in both a diagnostic sense, meaning capable of binding a molecule, the presence or absence of which one desires to know, and a therapeutic sense, capable of binding to a molecule associated with a disease.

Brief Summary Text (10):
The term "amplify" is used in the broad sense to mean creating an amplification product, which may include by way of example, additional target molecules, or target-like molecules, capable of functioning in a manner like the target molecule, or a molecule subject to detection steps in place of the target molecule, which molecules are created by virtue of the presence of the target molecule in the sample. In the situation where the target is a polynucleotide, additional target, or target-like molecules, or molecules subject to detection can be made enzymatically with DNA or RNA polymerases.

Brief Summary Text (12):
The term "contiguous" means an adjacent area of a molecule. By way of example, in the case of biological binding pairs, where a first ligand binds to a receptor target molecule, the area surrounding and adjacent to the first ligand is open and capable of binding to a second ligand contiguous to the first. In the context of

nucleic acid, where a first probe binds to an area of a larger nucleic acid target molecule, an adjacent mutually exclusive area along the length of the target molecule can bind to a second probe which will then be contiguous to the first. The target molecule acts as a template, directing the position of the first probe and the second probe. The term "substantially contiguous" is used in the functional sense to include spatial orientations which may not touch, may not abut, or may overlap, yet function to bring parts, areas, segments and the like into cooperating relationship.

Brief Summary Text (14):
The term "capture ligand" means a ligand capable of specifically binding with a capture antiligand associated with a support.

Brief Summary Text (17):
The term "reversible," in regard to the binding of ligands and antiligands, means capable of binding or releasing upon imposing changes which do not permanently alter the gross chemical nature of the ligand and antiligand. For example, without limitation, reversible binding would include such binding and release controlled by changes in pH, temperature, and ionic strength which do not destroy the ligand or antiligand.

Brief Summary Text (19):
Molecules of DNA consists of covalently linked chains of deoxyribonucleotides and molecules of RNA consists of covalently linked chains of ribonucleotides. Each nucleic acid is linked by a phosphodiester bridge between the 5'-hydroxyl group of the sugar of one nucleotide and the 3'-hydroxyl group of the sugar of an adjacent nucleotide. The terminal ends of nucleic acid are often referred to as being 5'-terminal or 3'-termini in reference to the terminal functional group. Complementary strands of DNA and RNA form antiparallel complexes in which the 3'-terminal end of one strand is oriented and bound to the 5'-terminal end of the opposing strand.

Brief Summary Text (20):
Nucleic acid hybridization assays are based on the tendency of two nucleic acid strands to pair at their complementary regions to form hybrids. The formation of such hybrids can be made to be highly specific by adjustment of the conditions (sometimes referred to as stringency) under which this hybridization takes place such that hybridization will not occur unless the sequences are precisely complementary. If total nucleic acid from the sample is immobilized on a solid support such as a nitrocellulose membrane, the presence of a specific "target" sequence in the sample can be determined by the binding of a complementary nucleic acid "probe" which bears a label. After removal of non-hybridized probe by washing the support, the amount of target is determined by the amount of detectable moiety present.

Brief Summary Text (22):
However, the sensitivity of such assays is limited by the number of labelled moieties which one may physically incorporate into the probe nucleic acid. In the case of radioactively-labelled probes, the practical limit of deletion is about 10.sup.4 target molecules. To achieve this sensitivity requires probes radioactive labels which have a very high energy and a very limited useful lifetime. The detection step, autoradiography, requires several days. Other labelling methods utilizing fluorescent, chemiluminescent, or enzymetic detection, although more rapid, usually do not exceed the sensitivity of radioactively-labelled probes. Since most organisms of clinical interest do not contain more than 50,000 copies of any nucleic acid suitable for use as a target, the utility of such methods is restricted to the detection of large numbers of organisms. The level of infectious agents in clinical specimens or foodstuffs, however, often does not exceed one to ten organisms.

Brief Summary Text (23):
One approach for the detection of low levels of DNA utilizes a DNA-dependent DNA polymerase to directly replicate the DNA target to increase its numbers to easily detectable levels. This approach is termed "polymerase chain reaction" (PCR). Saiki, R. K., Scharf, S., Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H. A., and

Arnheim, N., "Enzymatic Amplification of Beta-globin Genomic Sequences and Restriction Site Analysis for Diagnosis of Sickle Cell Anemia," Science 230: 1350-1354 (1985); Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B., and Erlich, H. A., "Primer-directed Enzymatic Amplification of DNA with a Thermostable DNA Polymerase," Science 239: 487-491 (1988); Erlich, H. A., Gelfand, D. H., and Saiki, R. K., "Specific DNA Amplification: Nature 331:461-(1988) and Mullis et al., European Patent application Nos. 200362 and 201184 (see also U.S. Pat. Nos. 4,683,195 and 4,683,202).

Brief Summary Text (24):
In practice, PCR is limited by the requirement that the target for amplification be DNA (as opposed to RNA), and by the occurrence of false positives generated by hybridization of probes to homologous sites in non-target DNA which fortuitously generate similar replication products. Moreover, although target DNA may be detected with very high sensitivity, the numbers of targets present in the sample is difficult to determine without adding significantly to the complexity of the assay. Since the number of infectious agents is often important in evaluating the treatment protocol for disease, this amplification approach is disadvantageously limited because it provides qualitative rather than quantitative results.

Brief Summary Text (26):
An autocatalytically replicatable RNA-probe construct may be employed in a sandwich hybridization assay, such as that described by Ranki, et al., U.S. Pat. No. 4,563,419; Soderlund, G. B., U.S. Pat. No. 2,169,403; Stabinsky, U.S. Pat. No. 4,751,177; and Syvanenen, et al., Nucl. Acids Res. 14:5037-5048. In the event the target is present and probe has hybridized to target, the autocatalytically replicatable RNA associated with the probe is replicated to generate amounts of RNA which may be easily detected by a variety of means (for example, by fluorescence using a dye such as ethidium bromide or propidium iodide). Since the MDV-1 RNA template for Q.beta. replicase is doubled in number every 20 seconds in vitro, an exponential increase (estimated to be a billion-fold) in the number of RNA molecules occurs within a few minutes at a single temperature. The autocatalytic reaction proceeds at an exponential rate until the number of autocatalytically replicatable RNA molecules exceeds the number of active enzyme molecules in the reactions. After that point, the amount of autocatalytically replicatable RNA increases linearly with time. As a consequence, in reactions given a sufficient period of time to reach this linear phase (for example 15 minutes for 100 molecules), the amount of amplified product RNA will be directly related to the logarithm of the number of autocatalytically replicatable RNAs initially added (Lizardi et al., supra). Since the initial number of autocatalytically replicatable RNA probes is proportional to the amount of target, the amount of target present in the sample being examined may be quantitated over a very wide range.

Brief Summary Text (28):
In another approach, the probe sequence may be incorporated within the sequence of the replicatable RNA (Lizardi et al., supra). However, the probe sequence is viewed as foreign by the enzyme and affects the ability of the RNA to be efficiently replicated, or is spontaneously deleted during replication. Deletion events affect the rate of replication and occur randomly with time. When deletion events occur, the level of the RNA products obtained in the linear phase of the amplification cannot be used to assess target level.

Brief Summary Text (30):
The discussion thus far has focused on signal generation. Signal generation which is related to the presence of target is very desirable. Signal generation which is not related to target, referred to as background is undesirable. By way of example, a single autocatalytically replicatable RNA molecule in the presence of Q-Beta replicase and reaction conditions, will initiate the production of copies at an exponential rate. In the event such single autocatalytically replicatable RNA is associated with a probe, which probe is bound to target, the exponential replication is a true positive detection. In the event such single autocatalytically replicatable RNA is not associated with a probe, or if associated with a probe and such probe is not associated with target, the exponential replication is a false positive or constitutes background from which true signal must be differentiated. The presence of background limits the sensitivity of assays at low target

[redacted]

concentrations. Target induced signal must be significantly greater than background in order for assays to be considered reliable.

Brief Summary Text (31):
One form of background, in affinity assays, occurs when the probe having a label associates with molecules other than target, and is carried through to detection. This type of background is often associated with non-specific binding of probe to supports.

Brief Summary Text (32):
One approach to reducing this non-specific binding background employs a method by which the target-probe complex is reversibly bound to the support ("reversible target capture"). After hybridization and immobilization, the complex is eluted from the support, which is then discarded with the non-specifically bound probe. The target-probe is then recaptured on fresh support. This process may be repeated several times to produce a significant reduction in the amount of non-hybridized probe (see Collins, European Patent Application No. 87309308.2).

Brief Summary Text (37):
The present invention features means for the control and amplification of autocatalytically replicatable molecules for diagnostic and therapeutic purposes. One embodiment of the present invention features a composition of matter. The composition of matter comprises a first nucleic acid having a first section and a second section. The first section is capable of autocatalytic replication under reaction conditions as part of the first nucleic acid, which includes the second section. The second section, positioned at one of the ends of the first section, is capable of assuming a bound position with a target.

Brief Summary Text (40):
A further embodiment of the present invention features a first nucleic acid having an inhibitory element and a first section, and a second section. The first section is capable of active autocatalytic replication under reaction conditions when the first section is separated from the inhibitory element and is inactive when such first section is part of the first nucleic acid integral with the inhibitory element. The second section has sequences which are capable of interacting with release means to separate the first section from the inhibitory element. The first nucleic acid is capable of assuming a bound position with a target, in which said second section is capable of interacting with release means.

Brief Summary Text (43):
A further embodiment features ligand and antiligand systems as inhibitory elements for inactivating the first section of the first nucleic acid. By way of example, ligands which are capable of binding to or interacting with the first section of the first nucleic acid may be capable of rendering the first section incapable of autocatalytic replication. Inhibitory elements utilizing ligand systems allow activation to occur following a cleavage event as a result of the destablization of the binding of the ligand system to the MDV-1 like sequences or activation may be due to the release of the first section to the extent that it is able to assume an active tertiary structure, or activation may be due to interactions with the enzyme. Inhibitory elements for inactivating the first section may include ligands and antiligand systems such as biotin-avidin or complementary nucleic acid sequences positioned in cooperating relationship on the first nucleic acid, antibody-antigen interactions and protein binding interactions.

Brief Summary Text (44):
One embodiment of the present invention features nucleotide sequences which are capable of interacting with the first section, rendering the first section inactive. One embodiment features sequences capable of interacting with MDV-1 like sequences in the first section in approximately the 81 to 126 MDV-1 nucleotide region. The interaction may include binding directly to the region but is not necessarily limited to such binding. The interaction may also include shielding the region from interaction with the enzyme Q-beta replicase, interfering with the enzyme, and distorting the tertiary structure of the region. A preferred sequence of nucleotides includes the nucleotides 5'-UUYRC-3' (SEQ ID NO:1), where Y represents any pyrimidine nucleotide and R is any purine nucleotide. One embodiment features

inhibitory sequences wherein Y is U and R is A.

Brief Summary Text (46):
In the specific case where the target is RNA, release means may be a small DNA oligonucleotide (for example, six nucleotides) complementary to a portion of the second section sequence of the first nucleic acid. In this case, the cleavage is ideally effected by the addition of RNase H (which acts to cleave RNA in RNA:DNA heteroduplexes) to the solution in contact with the support bearing the complex. Naturally, the means for capturing the RNA target should Ideally avoid generation of such heteroduplexes in order for the cleavage event to be specific. For example, a biotinylated RNA complementary to another portion of the target RNA may be conveniently captured upon an immobilized streptavidin support.

Brief Summary Text (47):
In the specific case where the target is DNA, digestion of the sequence extension of hybrids with target may be effected directly by the addition of RNase H, without the requirement for a second probe bearing such an oligonucleotide.

Brief Summary Text (50):
In one embodiment, the fourth section and second section require the presence of target which contributes nucleic acid sequences to form the ribozyme. The requirement for specific sequences in the target to form a ribozyme facilitates a further reduction in background. Signal can not be generated without target.

Brief Summary Text (52):
As used above, the letter X generally represents target, and X.sup.1 represents a first target region having one or more nucleotides which form a ribozyme with the first nucleic acid, X.sup.2 represents a terminal nucleotide of X.sup.1 or a second target region and X.sup.3 represents a terminal nucleotide of X.sup.1 or a third target region. The letter P generally represents the first nucleic acid, and P.sup.1 represents a first section, which section is capable of active autocatalytic replication when the first section is separated from an inhibitory element and inactive when the inhibitory element is integral with the first nucleic acid, in the presence of autocatalytic reaction conditions. The letter P.sup.2 represents a second section of first nucleic acid, which section contributes one or more nucleotides to form a ribozyme. The letter P.sup.3 represents an inhibitory element associated with the first nucleic acid. The letter P.sup.4 represents a fourth section capable of contributing one or more nucleotides to form a ribozyme.

Brief Summary Text (53):
In one embodiment which features a structure which resembles a "hammerhead" ribozyme, X.sup.1 and P.sup.4 are mutually exclusive and comprise one of the group of sequences 5'-MGAAAK-3' (SEQ ID NO:2), and 5'-J'CUGANGAM'-3' (SEQ ID NO:3), P.sup.2 comprises the sequences 5'-K'UWJ-3'(SEQ ID NO:4), wherein the letter N represents a nucleotide selected from the group of nucleotides comprising A, G, U and C. The letter W represents C or A. The letters J, J', K, K', M and M' each represent four or more nucleotides. The nucleotides of J and J', are complementary, as are the nucleotides of K and K' and the nucleotides of M and M'. Such complementarity is believed to provide stability and alignments for the ribozyme structure. Complementarity among nucleotide groups within sections and areas of the same nucleic acid, such as K' and J of second section P.sup.2, J' and M' of fourth section P.sup.4, allows such sections to form "stem" loops which open only on interaction with target, rendering such first nucleic acid incapable of forming ribozyme structures without specific target interaction. Such groups of nucleotides may also incorporate inhibitory sequences which interact with the first section.

Brief Summary Text (54):
The inhibitory element, P.sup.3, can be any moiety capable of inhibiting autocatalytic replication. Preferably, the inhibitory element is a nucleic acid. In which case, P.sup.3 can be any sequence of nucleotides. However, for diagnostic and therapeutic purposes, it is useful to have greater specificity to target than the sequences of M, M', K and K' may provide. The inhibitory sequences can be sequences capable of assuming a bound position to target at target region X.sup.1.

Brief Summary Text (55):

[redacted]

A further embodiment of the present invention features a first nucleic acid and a second nucleic acid which in the bound position to target form a ribozyme. One such structure is described by the Formula II below: ##STR2##

Brief Summary Text (56):
As used above, the letter X generally represents target, X.sup.1 represents a first target region, and X.sup.2 represents a terminal nucleotide of X.sup.1 or a second target region, and X.sup.3 represents a terminal nucleotide of X.sup.1 or a third target region. The letter P generally represents the first nucleic acid, P.sup.1 represents the first section which section is capable of active autocatalytic replication when the first section is separated from the inhibitory element and inactive when the inhibitory element is integral with the first nucleic acid and first section, in the presence of autocatalytic reaction conditions. The letter P.sup.2 represents a second section having one or more nucleotides which are capable of participating ribozyme formation. The letter P.sup.3 represents an inhibitory element. The letter P.sup.4 represents a terminal nucleotide of P.sup.1 or a fourth section capable of contributing sequences which participate in ribozyme formation with P.sup.2 and R.sup.1. As used above the letter R generally represents the second nucleic acid, and R.sup.1 represents a first area capable of having one or more nucleotides which participate in ribozyme formation. The letter R.sup.2 represents a terminal nucleotide of R.sup.1 or represents a second area of the second nucleic acid capable of assuming a bound position with respect to target region X.sup.1. At least one of R.sup.1, R.sup.2, P.sup.2, P.sup.3, are capable of assuming a bound position with target.

Brief Summary Text (57):
In one embodiment, the structure formed resembles a "hammerhead" ribozyme. In which case, the letter P.sup.2 represents the sequence, 5'-K'UWJ-3' (SEQ ID NO:4), X.sup.1 and R.sup.1 are mutually exclusive and represent one of the group of sequences 5'-MGAAAK-3' (SEQ ID NO:2), and 5'-J'CUGANGAM'-3' (SEQ ID NO:3), wherein N is one of the nucleotides U, G, A and C. The letter W represents C or A. The letters J, J', K, K', M and M' each represent four or more nucleotides. The nucleotides of J and J' are complementary as are the nucleotides of K and K' and the nucleotides of M and M'. P.sup.4 is a terminal nucleotide of P.sup.1.

Brief Summary Text (58):
Complementarity between nucleotide groups within an area or section, such as K' and J of second section P.sup.2, and J' and M' of first area R', allows such sections and areas to form "stem" loops which open up only on interaction with target, rendering such first nucleic acid incapable of forming a ribozyme structure without specific target interaction. Such areas may also incorporate inhibitory sequences which interact with the first section.

Brief Summary Text (60):
In one embodiment, the structure formed resembles a "hairpin" ribozyme. In which case, P.sup.2 represents the sequences 5'-FNGUCQ-3' (SEQ ID NO:5). The area represented by R.sup.1 comprises the sequences 5'-Q'AGAAF'ACCAGAGAAACACACGUUGUGGUAUAUUACCUGGUA-3' (SEQ ID NO:6). At least one of R.sup.2 and P.sup.3 are capable of assuming a bound position to target at X.sup.1, X.sup.2 or X.sup.3. The letter Q and Q', F and F' each represent four or more nucleotides. The letter N represents one of the nucleotides U, G, A, C. The nucleotides of Q and Q' are complementary, as are the nucleotides of F and F'. At least one of P.sup.3 or R.sup.2 are capable of assuming a bound position to target. The inhibitory element binding at target region X.sup.3 and the second area of the second nucleic acid assuming a bound position at target region X.sup.2.

Brief Summary Text (61):
Complementarity between nucleotide groups within an area or section, such as F and Q of second section P.sup.2, and F' and Q' of first area R', allows such sections and areas to form "stem" loops which open up only on interacting with target, rendering such first and second nucleic acids incapable of forming a ribozyme without specific target interaction. Such areas may also incorporate inhibitory sequences which interact with the first section.

Brief Summary Text (62):

As described with respect to Formula I, and now with respect to Formula II, greater specificity is obtained for target where the inhibitory element P.sup.3 is capable of assuming a bound position with target. In systems described with respect to Formula II, utilizing a first nucleic acid and a second nucleic acid, greater specificity can be obtained where R.sup.2 is capable of assuming a bound position with target.

Brief Summary Text (63):
The characteristic inability of the autocatalytically replicatable sequences to replicate efficiently until associated probes are specifically bound to target has led to descriptions of such compositions, as applied in diagnostics, as "smart probes." Smart probes provide reduction in background because they produce less or no signal until bound to target.

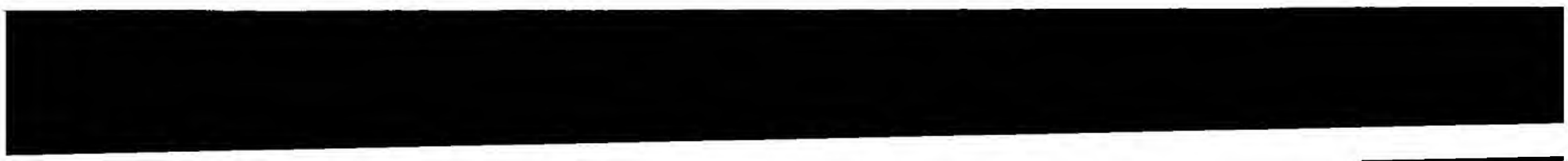
Brief Summary Text (64):
Compositions of the present invention have features of smart probes. Such smart probes have application in diagnostics, and can be used in conjunction with a variety of methods for background reduction such as sandwich assays and reversible target capture. However, the inherent low background of the compositions of the present invention may allow the compositions to be used in systems without a sandwich format and without reversible target capture.

Brief Summary Text (65):
One composition of the present invention features a first nucleic acid having a first section, and a second section, and inhibitory element and a support means. Each section has a 5' end and a 3' end. The support means is capable of binding to a support or is associated with a support. The first section is capable of active autocatalytic replication when the first section is separated from the inhibitory element and is inactive when the inhibitory element is associated with the first section. The inhibitory element is associated with the first section through the second section. The second section is capable of forming a ribozyme in the presence of target, which ribozyme cleaves the first section from the ligand under ribozyme reaction conditions.

Brief Summary Text (67):
As used above, the letter X generally represents target, X.sup.1 represents a first region, and X.sup.2 represents the terminal nucleotide of X.sup.1 or X.sup.2 represents a second region, X.sup.3 represents the terminal nucleotide of X.sup.1 or represents a third target region. The letter P generally represents a first nucleic acid, P.sup.1 represents a first section capable of active autocatalytic replication when the first section is separated from the inhibitory element, and inactive when integral with the inhibitory element. The letter P.sup.2 represents a second section capable of forming a ribozyme in the presence of the target. The letter P.sup.3 represents an inhibitory element such as, without limitation, nucleotide sequences capable of interaction with the first section, or such nucleotides which by virtue of their number, inhibit autocatalytic replication. The letter P.sup.4 represents the terminal nucleotide of the first section P.sup.1, or represents a fourth section capable of participating in the formation of a ribozyme. Support means, generally represented by the letter S, is any conventional or retrievable support, or a ligand capable of binding to such support. Support means may constitute inhibitory element, or inhibitory element may be part of support means.

Brief Summary Text (68):
One embodiment, which features a ribozyme which resembles "hammerhead" structure, P.sup.2 represents the sequences 5'-K'UWJ-3' (SEQ ID NO:4), X.sup.1 and P.sup.4 are mutually exclusive and are selected from the groups of sequences 5'-MGAAAK-3' (SEQ ID NO:2) and 5'-J'CUGANGAM'-3' (SEQ ID NO:3) wherein N is one of the bases U, G, A, and C. The letter W represents C or A. The letters J, J', K, K', M and M' each represent four or more nucleotides. The nucleotides of J and J' are complementary, as are the nucleotides of K and K', and the nucleotides of M and M'.

Brief Summary Text (69):
The composition may also comprise a second nucleic acid which cooperates with the first nucleic acid to form a ribozyme in the presence of target. Such a composition is described by the Formula IV set forth below: ##STR4##



Brief Summary Text (70):
As used above, the letters X.sup.1, X.sup.2, X.sup.3, S, P.sup.4, P.sup.3, P.sup.2 and P.sup.1 are consistent with the description immediately above. The letter R generally represents a second nucleic acid having a first area and a second area. The first area represented by the letter R.sup.1, is capable of forming a ribozyme with the second section P.sup.2 of the first nucleic acid. The letter P.sup.4 represents the terminal nucleotide of P.sup.1 or represents a fourth section to which contributes sequences to form a ribozyme. The second section is represented by the letter R.sup.2, is the terminal nucleotide of R.sup.1 or represents an area of the second nucleic acid capable assuming a bound position to the target at X.sup.3.

Brief Summary Text (71):
In one embodiment which resembles a "hairpin" ribozyme, P.sup.2 represents the nucleotide sequences 5'-FNGUCQ-3' (SEQ ID NO:5), R.sup.1 represents the nucleotide sequences 5'-Q'AGAAF'ACCAGAGAAACACACGUUGUGGUAUAUUACC UGGUA-3' (SEQ ID NO:6). The letters Q, Q', F and F' each represent four or more nucleotides. The nucleotides of Q and Q' are complementary as are the nucleotides of F and F'. P.sup.4 is the terminal nucleotide of P.sup.1. At least one of the sections or areas of the first nucleic acid and second nucleic acid are capable of assuming a bound position. The second area of the second nucleic acid assuming a bound position at target third region X.sup.3 and inhibitory element P.sup.3 assuming a bound position with target at target second region X.sup.2.

Brief Summary Text (72):
In one embodiment, which features a structure which resembles a "hammerhead" ribozyme, P.sup.2 represents the sequences 5'-K'UWJ-3' (SEQ ID NO:4), X.sup.1 and R.sup.1 are mutually exclusive and represent one of the groups of sequences 5'-MGAAAK-3' (SEQ ID NO:2) and 5'-J'CUGANGAM'-3' (SEQ ID NO:3) wherein N is one of the bases U, G, A and C. The letter W represents C or A. The letters J, J', K, K', M and M' each represent four nucleotides. The nucleotides of J and J' are complementary, as are the nucleotides of K and K' and the nucleotides of M and M'. P.sup.4 is the terminal nucleotide of P.sup.1.

Brief Summary Text (74):
The support means in formulas III and IV allows the target nucleic acid complex to be separated from debris, reagents, and other nucleic acid which may be present in the sample. The first section of the first nucleic acid is inactive, unless in the presence of target it is separated or cleaved, minimizing or eliminating background.

Brief Summary Text (75):
By way of example, support means may include a biotin group for capture upon a support derivatized with avidin or streptavidin, a fluorescein group for capture upon a support bearing immobilized antibodies to fluorescein, a poly A tail for capture upon a support bearing immobilized oligo or poly dT and a binding site for the coat protein of bacteriophage R17 for capture upon a support bearing the coat protein.

Brief Summary Text (76):
A number of means may be employed to associate a ligand with the first nucleic acid. By way of example, where the support means is 3' to the site of cleavage, biotin, fluorescein, proteins, antibodies and antigens may be associated by one of several methods. These include, but are not limited to: (1) ligating a small RNA or DNA oligonucleotide produced synthetically and containing one or more biotins to the 3' terminus with T4 RNA ligase (2) addition of an RNA tail to the 3' terminus with E. coli poly A polymerase using biotinylated ribonucleoside triphosphates (3) periodate oxidation of the 3' terminal residue, followed by coupling of the dialdehyde product to a biotinylated molecule bearing a primary amine followed by reduction, and (4) hybridization of a biotinylated complementary RNA to a region distal (e.g.-3' to) the target-binding region of the midivariant probe.

Brief Summary Text (77):
Turning now to methods of the present invention which relate to diagnostics, the methods of the present invention feature each of the composition herein described.

This discussion will focus on selected compositions by way of example, without limitation. One embodiment of the present invention includes a method for detecting the presence of a target nucleic acid in a sample comprising the steps of contacting a sample with a first nucleic acid which nucleic acid has a first section and a second section. The first section is capable of autocatalytic replication under reaction conditions. The first section has a 3' end and a 5' end. The second section is positioned at one of the ends of the first section and is capable of assuming a bound position in the presence of binding conditions with target. The method further includes the step of imposing binding conditions on the sample to allow the first nucleic acid to form a target-first nucleic acid complex. Unbound first nucleic acid is separated from the sample. The imposition of reaction conditions for autocatalytic replication on the sample allows the sample to be monitored for the presence of the autocatalytic reaction product which will be formed in the presence of the first nucleic acid, which reaction product is indicative of the presence of the target.

Brief Summary Text (78):
A further embodiment of the present invention includes a first section which has sequences which are substantially identical to MDV-1 and is capable of autocatalytic replication in the presence of the enzyme Q-beta replicase. Preferably, in imposing reaction conditions, which reaction conditions include contacting the sample with the enzyme Q-beta replicase, an enzyme is used which has no endogenous activity. Surprising and unexpectedly, when such nucleic acid compositions with first sections having sequences substantially identical to MDV-1, and second sections capable of binding to target, are used with the enzyme Q-beta replicase, it is possible to discern, the presence of target from what had been previously characterized as "unprimed" activity.

Brief Summary Text (79):
A further embodiment includes a method for detecting the presence of target nucleic acid in a sample comprising the step of contacting the sample with a first nucleic acid which nucleic acid has a first section, and second section and is associated with an inhibitory element. Each section has a 5' end and a 3' end. The first section is capable of active autocatalytic replication under reaction conditions when the first section is separated from the inhibitory element and is inactive when integral and associated with the inhibitory element. The second section is positioned at one of the ends of the first section and is capable of cleavage upon interaction with release means, to separate the first section from the inhibitory element. At least one section or the inhibitory element of the first nucleic acid is capable of assuming a bound position to target in the presence of binding conditions. Imposition of binding conditions on the sample in the first nucleic acid allows the formation of a target first nucleic acid complex in the presence of target. The sample is contacted with release means to separate the first section from the inhibitory element. Imposition of autocatalytic replication reaction conditions on the sample, allows the formation of an autocatalytic reaction product in the presence of the first nucleic acid. The sample is monitored for the presence of the autocatalytic reaction product, which autocatalytic reaction product is indicative of presence of target.

Brief Summary Text (82):
One embodiment of the present invention features a ribozyme formed by the first nucleic acid and the target. The method incorporates additional "smartness" due to the need for specific target sequences which do not allow a ribozyme to form unless the first nucleic acid is bound to target. One embodiment features a ribozyme which requires target sequences to form the ribozyme.

Brief Summary Text (83):
In a further embodiment, the method includes the formation of a ribozyme comprising a first nucleic acid and a second nucleic acid, both of which can be made to interact with target in order to form stable ribozyme structures.

Brief Summary Text (84):
In one structure resembling a "hammerhead," the target contributes the sequences 5'-MGAAAK-3' (SEQ ID NO:2), a first nucleic acid or second nucleic acid has a section or an area which contributes the sequences 5'-J'CUGANGAM'-3' (SEQ ID NO:3),

and first nucleic acid has a section which contributes the sequences 5'-K'UWJ-3' (SEQ ID NO:4). As used herein, N is one of the four nucleotides U, G, A and C. The letter W represents C or A. The letters J, J', K, K', M and M' each represent four or more nucleotides. The nucleotides of J and J' are complementary, as are the nucleotides of K and K' and the nucleotides of M and M'.

Brief Summary Text (87):
Additional embodiments of the present invention relate to compositions of matter which are useful as ribozymes. One embodiment of the present invention features a first nucleic acid and a second nucleic acid in which the first nucleic acid has a first section and a second section. Each section having a 5' end and a 3' end. The first section has less than all the sequences performing a ribozyme and the second section is capable of assuming a bound position with a target and is positioned at one of the ends of the first section. The second nucleic acid has a first area and a second area, each area having a 5' end and a 3' end. The first area has sequences which complete the formation of a ribozyme when the first area is held in a cooperating relationship with the first section of the first nucleic acid. The second area is capable of assuming a bound position to target in which the first area is held in cooperating relationship.

Brief Summary Text (88):
In one embodiment of the present invention, the ribozyme resembles a "hammerhead." In one "hammerhead" configuration, the target contributes the sequences 5'-MGAAAK-3' (SEQ ID NO:2) for the completion of the ribozyme. The first section of the first nucleic acid includes the sequences 5'-J'CUGANGAM'-3' (SEQ ID NO:3) wherein the letter N is used to represent a nucleotide selected from the group of nucleotides consisting of A, G, U or C. The first area of the second nucleic acid contributes sequences 5'-K'UWJ-3' (SEQ ID NO:4). The letter W represents C or A. The letters J, J', K, K', M, and M' each represent four or more nucleotides. The nucleotides of J and J' are complementary, as are the nucleotides of K and K', and the nucleotides of M and M'.

Brief Summary Text (90):
Further embodiments of the present invention feature methods of making ribozymes. In one embodiment, the method includes binding a first and second nucleic acid to a target in cooperating relationship. The first nucleic acid has less than all sequences for ribozyme activity. The second nucleic acid has less than all sequences necessary for all ribozyme activity and in a bound position, form a ribozyme.

Brief Summary Text (92):
In order to facilitate an understanding of the invention, the discussion with respect to ribozyme compositions and methods of making ribozymes has focused on nucleic acid probes. However, sections and areas of the first and second nucleic acid which bind to a target can be substituted with any ligand which binds to target to position sections and areas which form a catalytic sequence group. Embodiments of the present invention featuring the formation of ribozymes encompass the use of probes in a broad sense including immunodiagnostic agents, such as antibodies and antigens, and other biological binding pairs such as, drugs and drug receptor sites, enzymes and enzyme substrates.

Drawing Description Text (2):
FIG. 1, consisting of FIG. 1A and FIG. 1B, illustrates a method of determining the presence of a target molecule which target molecule is capable of contributing sequences to form a ribozyme in a "hammerhead" configuration.

Drawing Description Text (3):
FIG. 2 generally depicts an alternative "hammerhead" ribozyme configuration.

Drawing Description Text (4):
FIG. 3 generally illustrates a first and second nucleic acid bound to target forming a ribozyme in a "hairpin" configuration.

Drawing Description Text (5):
FIG. 4 illustrates a structure of a ribozyme with an autocatalytic replicating section associated with a support, in the presence of target.

Detailed Description Text (2):
To facilitate discussion of the present invention, reference will be made to the figures which describe methods and compositions for the detection of target. However, those skilled in the art will readily recognize that the methods and compositions of the present invention have value and application in therapeutics to deliver molecules to sites for activation.

Detailed Description Text (3):
Turning now to FIG. 1, which consists of FIGS. 1A and 1B, a method of detecting the presence of a target molecule is generally depicted.

Detailed Description Text (4):
As illustrated, the target molecule is a nucleic acid generally designated by the number 11. The target molecule is associated with a capture bead 13 by means of a capture ligand 15 which is hybridized to a capture nucleic acid, generally described by numeral 21. The capture nucleic acid 21 has a capture antiligand section generally described by the numeral 23 and a probe section 25 capable of hybridizing to the target 11. The capture nucleic acid is illustrated in a hybridized position to the target 11.

Detailed Description Text (5):
The target 11 has a first segment 26 and a second segment 27 and a third section 29. The first segment 26 and third segment 29 define binding sites. The second segment 27 has sequences which participate in the formation of a ribozyme.

Detailed Description Text (6):
A first nucleic acid 31 is illustrated bound to the target 11. The first nucleic acid 31 has a first section 33, second section 35, a third section 37 (as can be seen in FIGS. 2, 3, and 4) and a fourth section 41. The first section 33 is capable of autocatalytic replication. As illustrated, the sequences of the first section 33 includes sequences that are substantially identical to the sequences of MDV-1. The second section 35 has sequences which are able to participate in the formation of a ribozyme. The third section 37 is capable of binding to the target at the target segment 27. The fourth section 41 includes at least four base sequences 43 capable of binding the target at target third segment 29, and contributes sequences to the formation of the ribozyme. The sequences of the fourth section 41 which participate in ribozyme formation are generally designated by the numeral 45 and those sequences capable of binding target are designated 43. Upon imposition of ribozyme reaction conditions in the presence and target, the first nucleic acid 31 is capable of cleavage in second section 35 (see FIGS. 2 and 3) at the position designated as 39.

Detailed Description Text (8):
Thus, in FIG. 1B, the first section 33 is shown as a distinct part from the rest of the first nucleic acid 31. The few remaining nucleotides of the fourth section 41 which are capable of binding to the second nucleic acid at 29, are unable to form a stable hybrid under the conditions present in the sample. The first section 33 becomes disassociated from the target-probe complex and receptive to autocatalytic replication reaction conditions.

Detailed Description Text (9):
Upon imposition of autocatalytic reaction conditions, the first section 33 is replicated on an exponential basis initially. The fourth section 41 need not, and normally is not, replicated. Upon saturation of the enzyme Q-beta replicase with the first section 33 and its copies, the first section 33 and its copies are replicated in a linear fashion. In a diagnostic assay, the sample is monitored for the presence of the autocatalytic reaction product which product is indicative of the presence of target 11. Additionally, if one monitors the concentration of the autocatalytic reaction product, the concentration of target 11 can be calculated on the basis of the amount of autocatalytic reaction product produced in time.

Detailed Description Text (10):
FIG. 2 describes an alternative "hammerhead" ribozyme structure formed by target 11, and a first nucleic acid 31 and a second nucleic acid 41. The ribozyme structure depicted in FIG. 2 has a cleavage site 39 is positioned towards the 5' end of the

first nucleic acid 31.

Detailed Description Text (11):
The target 11 includes a first segment 25, second segment 27 and a third segment 29. A first nucleic acid 31 includes a first section 33, a second section 35 and a third section 37. The first section includes base sequences which are substantially identical to MDV-1 and are capable of autocatalytic replication when removed from the third section 37. The second section 35 is capable of contributing sequences which form a ribozyme. The third section 37 is capable of binding to the target 11 at the third segment 29.

Detailed Description Text (12):
A second nucleic acid 41 includes a first area 43 and a second area 45. The first area 43 is capable of binding to the target 11 at first segment 25. The second area 45 of the second nucleic acid 41 is capable of contributing sequences to form a ribozyme.

Detailed Description Text (13):
As illustrated, the target 11 itself contributes sequences to the ribozyme, which sequences include the sequences 5'-GAAA-3' (SEQ ID NO:7). Individuals skilled in the art will recognize that the designation of target 11 and second nucleic acid 41 is somewhat arbitrary, depending on the molecule of interest and what sequences are available in such molecule. It is much more probable that a particular molecule of interest will have the sequences 5'-GAAA-3' (SEQ ID NO:7), than the sequences 5'-CUGANGA-3' (SEQ ID NO:8). In a bound position to the target 11 the first nucleic acid 31 and the second nucleic acid 41 form a ribozyme which upon imposition of ribozyme reaction conditions cause cleavage at position 39, releasing the first section 33 from the third section 37. The first section 33, removed from the third section 37 is capable of autocatalytic replication upon imposition of autocatalytic replication reaction conditions.

Detailed Description Text (14):
Turning now to FIG. 3, a target is illustrated generally depicted as 11 in which a first nucleic acid 31 and a second nucleic acid 41 are bound. The first nucleic acid 31 and the second nucleic acid 41 create a "hairpin" ribozyme structure. The target 11 includes a first segment 27 and a second segment 29 each having a 5' end and a 3' end. The first nucleic acid 31 includes a first section 33, a second section 35 and a third section 37. The first section 33 is capable of autocatalytic replication in the presence of autocatalytic reaction conditions and upon separation from the third section. The second area 35 capable of contributing sequences to a ribozyme structure. The third section 37 is capable of binding to the first segment 27 of the target 11.

Detailed Description Text (16):
To facilitate synthesis and cloning manufacturing, each area of the second nucleic acid 41 is linked by a conventional phosphodiester bridge 5' to 3' or 3' to 5'. The first area 43 is capable of assuming a bound position to the second segment 29 of the target 11. The second area 45 of the second nucleic acid 41 is capable of forming a "hairpin" ribozyme with the second section 35 of the first nucleic acid 31. The second area 45 of the second nucleic acid 41 and the second section 35 of the first nucleic acid 31 define a ribozyme having a cleavage site 39 on the first nucleic acid 31.

Detailed Description Text (18):
FIG. 4 illustrates compositions similar to that illustrated in FIG. 3. A target is illustrated generally depicted as 11 in which a first nucleic acid 31 and a second nucleic acid 41 are bound. The first nucleic acid 31 and the second nucleic acid 41 create a "hairpin" ribozyme structure. The target 11 includes a first segment 27 and a second segment 29 each having a 5' end and a 3' end. The first nucleic acid 31 has a first section 33, a second section 35 and a third section 37. The first section 33 is capable of autocatalytic replication in the presence of autocatalytic reaction conditions and upon separation from the third section. The second area 35 capable of contributing sequences to a ribozyme structure. The third section 37 is capable of binding to the first segment 27 of the target 11, and is associated with support means 51. Support means 51 is a biotin which is capable of binding to avidin 53

bound to support 13.

Detailed Description Text (20):
To facilitate synthesis and cloning manufacturing, each area of the second nucleic acid 41 is linked by a conventional phosphodiester bridge 5' to 3' or 3' to 5'. The first area 43 is capable of assuming a bound position to the second segment 29 of the target 11. The second area 45 of the second nucleic acid 41 is capable of forming a "hairpin" ribozyme with the second section 35 of the first nucleic acid 31. The second area 45 of the second nucleic acid 41 and the second section 35 of the first nucleic acid 31 define a ribozyme having a cleavage site 39 on the first nucleic acid 31.

Detailed Description Text (21):
After a complex of the target 11 with the first nucleic acid 31 and second nucleic acid 41 is formed, the first nucleic acid 31 is captured on support 13 through the biotin 51 and avidin 53. The support 13 is separated from the remaining solutions, which may include first nucleic acid 31 unbound to target 11, and first sections 33 which have dissociated from the first nucleic acid 31 through an event not mediated by target 11, to reduce background.

Detailed Description Text (30):
Two oligonucleotides are synthesized. The first of these advantageously contains the sequence 5'-CCCCTGANGA-3' (SEQ ID NO:10) followed by at least four nucleotides complementary to the sequence in the target RNA 5' to the 5'-GAAA-3' (SEQ ID NO:7) element and terminating in the sequence 5'-GATC-3' (SEQ ID NO:11). The second oligonucleotide ideally contains the sequence 5'-CCCGA-3' (SEQ ID NO:12) followed by at least 4 nucleotides of the sequence 3' to the 5'-GAAA-3' (SEQ ID NO:7) element in the target, except that deoxyribonucleotides replace the ribonucleotides of the target. This element is advantageously followed immediately by the element 5'-GGGG-3' (SEQ ID NO:13). Each of the oligonucleotides is phosphorylated on its 5' terminus by T4 polynucleotide kinase.

Detailed Description Text (36):
The cDNA clone as described in Example 1 is cleaved with the enzymes Apa I and Ear I, and the large fragment is ideally purified away from the small fragment described above. An oligonucleotide having the sequence 5'-CCCGA-3' (SEQ ID NO:12) followed by 4-50 nucleotides identical to the sequence immediately 3' to a 5'-GAAA-3' (SEQ ID NO:7) element in the target, in turn followed by the sequence 5'-GGCC-3' (SEQ ID NO:14) is synthesized by any of a number of methods familiar to those skilled in the art. This oligonucleotide is annealed and ligated to the large restriction fragment, and the resulting single stranded "gap" region rendered double-stranded by the action of a DNA-dependent DNA polymerase. This DNA is introduced into and propagated within bacteria. After purification from bacteria, it may be cleaved with the restriction endonuclease Sma I, and then used as a template in an in vitro transcription reaction utilizing bacteriophage T7 RNA polymerase.

Detailed Description Text (37):
The second sequence probe may be generated by constructing a synthetic DNA template for T7 RNA polymerase as described by Milligan et al. (Nucl. Acid. Res. (1987)15:8783-8798), one strand of this template starting at its 5' end with at least 4 nucleotides of the sequence from the region 5' to the 5'-GAAA-3' (SEQ ID NO:7) element in the target except that deoxyribonucleotides replace the ribonucleotides found in the target, followed by the sequence 5'-TCNTCAGGGGGCCCTATAG TGAGTCGTATTA-3' (SEQ ID NO:15) where N indicates any nucleotide having the sequence 5'-TAATACGACTCACTATAG-3' (SEQ ID NO:16). These two oligonucleotides preferably are mixed and transcribed in vitro as described by Milligan et al. (ibid). The product may be readily purified by any of a number of methods familiar to those acquainted with the art.

Detailed Description Text (43):
An assay for target nucleic acid utilizing a ribozyme probe.

Detailed Description Text (45):
One picomole (10.sup.-12 mol) of this RNA is hybridized to whole human blood from patients infected with human immunodeficiency virus type 1 (HIV-1), normal human

blood, or a sample to be assayed for the presence of HIV-1, in a sandwich hybridization assay such as described by Ranki et al. (ibid) in the presence of 2.5 M guanidine thiocyanate and one picomole of a synthetic deoxyribo-oligonucleotide having the sequence 5'-GGAAGCACATTGTACTGATATCTAAT CCCTGGTGGTCTCATA.sub.150 -3' (SEQ ID NO:21). With general reference to FIG. 1A the hybrid is bound to a solid support by hybridization of the dA150 tract in the oligonucleotide to immobilized polydeoxythymidine such as in the well of a polystyrene microtiter place (or a capture bead as shown in FIG. 1A) coated with dT.sub.3000 according to the methods of Collins et al., (U.S. Ser. No. 922,155 and CIP U.S. Ser. No. 136,920, fully incorporated herein by reference). The plate is incubated at 37.degree. C. for 30 minutes after which the contents, containing unbound probes, are aspirated and discarded, and the well repeatedly washed with a buffer containing 9.5M guanidine thiocyanate. The well is then washed with a buffer containing 90 mM Tris HCl (pH7.5), and 50 .mu.l of the same buffer except containing 14 mM MgCl.sub.2 introduced into the well and incubated at 50.degree. C. for 15 minutes to induce ribozyme cleavage. The contents of the well containing the cleaved and released probe (see FIG. 1B) are transferred to another well for replication. Five microliters of a solution containing 4 mM each ATP, GTP, CTP, and UTP, 10 .mu.lCi of .alpha.-.sup.32 P-CTP and 1 .mu.g of Q.beta. replicase are added and the plate incubated at 37.degree. C. for 25 minutes. Two microliters of the well contents are removed and added to 18 .mu.l of 95% formamide, 0.05% xylene cyanol, 0.05% bromphenol blue for halting replication. Five microliters of this are applied to the well of a denaturing 8% polyacrylamide gel, and the replicated RNAs resolved by electrophoresis until the xylene cyanol dye has migrated the length of the gel. An x-ray film is exposed to the gel for three hours and is then developed as the final step of detection. The larger RNA product of length equal to the number of nucleotides between the sequence corresponding to the unappended 5' terminus of the naturally-occurring MDV-1 RNA and the unappended 3' terminus of MDV-1 RNA and including the length of the sequence element inserted into the Hinf site such as described in Example 1 (in that case 10 nucleotides), indicates the presence of HIV-1 virus or its messenger RNA in the blood sample. It will also be readily recognized that by carefully timing the replication phase and comparing the results against a suitable control, correlation can be made between the amount of replicated RNA with the amount of target RNA in the original sample thereby providing quantitation results.

Detailed Description Text (48):
An assay for target HIV-1

Detailed Description Text (55):
These results provide practical guidance for using replicatable RNAs bearing 3' sequence extensions as probes for the sensitive detection of nucleic acids. By appropriate selection of additional sequences from the 3' end, RNA probes may be advantageously generated which have intrinsically greater sensitivity in the detection of target nucleic acid. Conversely, for some target nucleic acids which are present in an infectious agent at high levels (i.e.--ribosomal RNA, present at up to 50,000 copies per organism), additional sequences conferring relatively poor limits of detection may be utilized to advantageously avoid the background otherwise generated by the amplification of lower levels of non-specifically bound probes. For example, if reduced in number to levels at least an order of magnitude below the limit of detection using background reduction methods such as Collins (supra), non-specifically bound probes will be incapable of producing a detectable signal. Thus, the probes of the present invention comprising additional sequences from the 5' end may be used advantageously to reduce the cost, complexity, and frequency of false-positive reactions in such assays.

Detailed Description Text (58):
An important advantage issued from the present discovery which has additional application for smart probe systems which employ the target-directed cleavage of 5' or 3' sequence extensions, since such methods need not provide for the precise and complete removal of those sequences. For example and as set forth in U.S. Ser. No. 252,243, a probe may be generated bearing both 3' and 5' extensions whose hybridization to target produces a ribozyme structure which directs cleavage of the 5' extension from the RNA. A short 3' extension remains on the molecule.

Detailed Description Text (59):
A most preferred embodiment of the present invention is diagrammed in FIG. 4, wherein a probe molecule bearing an extension is hybridized to target in solution. Shortly before, after, or simultaneously therewith, a second oligonucleotide probe is hybridized to the target adjacent to the first probe. The second oligonucleotide probe is ideally coupled to a nuclease which requires a cofactor (for example, a divalent cation) for its activity. Following hybridization, the complex is captured on a solid support in a manner such as that described by Rankmi et al. (supra), Soderlund (supra), Stabinsky (supra), and/or Syvanenen (supra). After separating, such as by washing away, the bulk of non-specifically bound probes, the cofactor for nuclease activity is added. The nuclease, coupled to the terminus of the probe proximal to the first probe when hybridized to its target, cleaves the spacer on the first probe, thereby releasing a replicatable moiety into solution. As previously indicated, the third section may be advantageously selected to maximally inhibit replication until cleavage occurs. Other variations of this approach will become apparent. For example, such probe parts may include a replicatable RNA instead bearing a 5' extension and a nuclease-coupled probe in which the ribozyme sequence is present 5' to the probe sequence element. Any of a number of nucleases may be advantageously employed as the release means. For example, micrococcal nuclease may be used since it does not cleave MDV-1 RNA (Hill & Blumenthal (1983) Nature 301, 350-352). Corey & Schultz (1987) Science 238:1401-1403, teach the construction of such oligonucleotide-micrococcal nuclease conjugates. Any number of ribozymes with endonucleolytic cleavage activity, such as those described by Haselhoff & Gerlach (Nature 3343:585-591 (1988)), Uhlenbeck (1987) Nature 328, 596-600, Ruffner (1990) Biochemistry 29, 10695-10702, Cech (European pat. Appl. WO 88/04300, June 1988), Sharmeen et al. (1989) J. Virol. 63, 1428-1430; or Hampel & Tritz (1988) J. Cell. Biochem., Suppl. 12D, Abst. #N212, p.31, may be employed. Ribozymes possess a significant advantage in that the second probe bearing the ribozyme may be efficiently produced in a single step by transcription of a DNA oligonucleotide of appropriate sequence such as is described by Milligan (supra), thus reducing the cost and labor required to generate such reagents. Moreover, ribozymes tend to produce specific cleavage events, leading to a product RNA with defined replication properties.

Detailed Description Text (60):
In the specific case where the target is RNA, release means may be a small DNA oligonucleotide (for example, six nucleotides) complementary to a portion of the second sequence section of the first nucleic acid. In this case, the cleavage is ideally effected by the addition of RNase H (which acts to cleave RNA in RNA:DNA heteroduplexes) to the solution in contact with the support bearing the complex. Naturally, the means for capturing the RNA target should ideally avoid generation of such heteroduplexes in order for the cleavage event to be specific. For example, a biotinylated RNA complementary to another portion of the target RNA may be conveniently captured upon an immobilized streptavidin support.

Detailed Description Text (61):
In the specific case where the target is DNA, digestion of the sequence extension of hybrids with target may be effected directly by the addition of RNase H, without the requirement for a second probe bearing such an oligonucleotide.

Detailed Description Text (67):
were annealed and ligated into a MDV cDNA construct similar to that described by Lizardi (supra), which had been digested with EcoRI. This cDNA clone differed from those described by Lizardi et al. in that the internal insert encoded a binding site for the coat protein for phage R17. This cDNA was obtained from F. R. Kramer, Public Health Research Institute, N.Y. The oligonucleotides were ligated in the orientation such that upon subsequent digestion with EcoRI, cleavage occurred downstream of the oligonucleotide with respect to the promoter for T7 RNA polymerase. The digested plasmid was transcribed in vitro with T7 RNA polymerase under the conditions described by Mulligan et al. (supra), and the transcription product of correct length isolated by electrophoresis through polyacrylamide gels containing 8.3M urea.

Detailed Description Text (69):
Various numbers of formalin-fixed elementary bodies of Chlamydia trachomatis were

lysed in a solution of 9.0 mg/ml proteinase K (Boehringer Mannhein) and 1.6% Sarkosine (Sigma) at 65.degree. C. for 15 minutes in a final volume of 35 .mu.l. Thirty-five .mu.l of a buffer containing 340 ng/ml of tailed capture oligonucleotide and 100 ng/ml of the transcription product probe was then added and solution phase hybridization allowed to occur for 30 minutes at 37.degree. C. Fifty .mu.l of a 0.06% (w/v) suspension of oligo-dT derivitized magnetic beads prepared according to Collins (supra) in 4% BSA, 10 mM EDTA, 0.2% Sarkosine, 9.1M Tris-HCl pH8.0 and 0.05% bronopol was then added and incubated for an additional 5 minutes at 37.degree. C. to capture the target-probe hybrids on the beads.

Detailed Description Text (71):
The collected beads, freed of supernatant, were then resuspended in 50 .mu.l of a buffer containing 3.25M GuSCN, 65 mM EDTA, 0.04M Tris-HCl, pH7.0, 0.5% Sarkosine, and 0.5% BSA, and incubated at 37.degree. C. for 5 minutes to release the target-MDV probe-capture probe hybrids. The magnetic beads were collected as before, and the supernatants removed and transferred to a fresh set of tubes, each containing 50 .mu.l of a fresh bead suspension to recapture the hybrids as described above. These beads were washed three times in the same manner as the first set, the hybrids released and recaptured by a third set of beads. This set of beads was washed three times in the same manner, and additionally, three times with 0.2 ml of a solution containing 0.1M KCl, 1 mM EDTA, 10 mM Tris-HCl, pH8.0, 0.5% NP-40.

Detailed Description Text (80):
A library of template DNAs can be generated by synthesis of an oligonucleotide having in 5' to 3' order: (1) a short (for example, 40 nucleotides) sequence identical to target, (2) a short stretch (15 nucleotides) of random sequence, and (3) a sequence complementary to the 3'-most 30 nucleotides of MDV-1 RNA. The random sequence element may be generated with ease by addition of all four blocked nucleotides during synthesis of the oligonucleotide, a procedure easily accomplished with currently available automated synthesizers. A second oligonucleotide is then synthesized which is complementary to the nineteen 5'-most nucleotides of the first oligonucleotide. This is annealed to the first oligonucleotide and extended with a DNA-dependent DNA polymerase to produce a double-stranded DNA fragment. This fragment is then cleaved with the restriction enzyme Bst EII and the cleavage product ligated to a fragment of a MDV cDNA clone such as described by Lizardi (supra), previously cleaved with Bst EII. Two picomoles of the ligation product is then transcribed by T7 RNA polymerase to generate a population of RNAs containing 10.sup.12 different spacer sequences.

Detailed Description Text (83):
The sensitivity of detection of the cloned RNAs may be determined as follows. The plasmid DNAs from each clone is purified by standard methods. Each is restricted with a restriction enzyme immediately downstream of the cDNA and transcribed by T7 RNA polymerase to produce an RNA product devoid of vector sequence. This RNA is purified either by gel electrophoresis or by capture onto oligo dT-magnetic beads as in the method described in Example 2 with a DNA oligonucleotide of the same sequence as the target tailed with dA.sup.150 except that a second capture probe is omitted. Each of the purified transcripts is serially diluted in water and aliquots (10 .mu.l) added to Q.beta. reactions as described in Example 2. The reactions are allowed to run 30 min. at 37.degree. C., stopped, and the minimum number of probes required to observe product determined by the highest dilution producing fluorescence. This process is repeated for clones obtained from each round of the selection process.

Detailed Description Text (84):
It will be readily appreciated that the above process may also be applied to the isolation of RNAs which replicate efficiently when hybridized to target. This is simple performed by first hybridizing the population of RNAs to a synthetic target prior to the formation of initiation complexes. As will also be recognized, members of such a population-may contain molecules which replicate more efficiently when hybridized to target than when free. Such molecules will be preferred probes for use in hybridization assays since the molecules non-specifically carried through the hybridization process will be intrinsically less efficiently replicated.

Detailed Description Text (96):

[redacted]

To obtain a ribozyme probe for the RNA bearing the CAUUAC (SEQ ID NO:41) element, an oligonucleotide having the sequence: 5'-TACCAGGTAATATA CCACAACGTGTGTTTCTCTGGTATGATTCTCATTACGAGACAGCAGTACAAATGGCAGTATTCATCCACA ATTTTCCCTATAGTGAGTCGTATTAAT-3' (SEQ ID NO:44) was synthesized. This was annealed to an oligonucleotide of the sequence 5'-ATTAATACGACTCACTATAGGG-3' (SEQ ID NO:45) ("T7 Promoter-Primer", Promega Sciences, Madison, Wis.) and transcribed with T7 RNA polymerase under conditions described by Milligan et al. (ibid), and the product RNA purified by denaturing polyacrylamide gel electrophoresis. This yielded an RNA of the sequence: 5'GGGAAAAUUGUGGAUGAAUACUGCCAUUUGUACUGCUGUCUCGUAAUGAGAAUC AUACCAGAGAAACACACGUUGUGGUAUAUUACCUGGUA-3' (SEQ ID NO:46). When annealed to HIV target nucleic acid with the MDV probe bearing the CAUUAC (SEQ ID NO:41) inhibitory sequence element, the structure similar to that represented in FIG. 3 is obtained.

Detailed Description Text (97):
To test for the cleavage activity, 10fmol of .sup.32 P-labelled midivariant probe and 100fmol ribozyme probe were annealed to 1 ng of a synthetic HIV target RNA, and the hybrid complexes isolated by reversible target capture on magnetic particles, as generally described in GENE-TRAK Systems HIV assay, which assay is commercially available, except that only two rounds of capture and release were performed. After washing the final magnetic particles to which the complexes were immobilized with 0.3M KCl as described, the particles were resuspended 100 .mu.l 0.05M Tris-HCl, pH7.8, 15 mM MgCl.sub.2, and 0.5% NP-40, and incubated at 37.degree. C. for 15 minutes. The particles were removed from solution by placing in a magnetic field, and the supernatant removed and counted. Twenty seven percent of the cpm initially bound to the particles was released into solution, compared to less than one percent in a control reaction in which the ribozyme probe was omitted.

Detailed Description Text (100):
Specific placement of an affinity ligand on the portion of a cleavable midivariant probe which is distal to the cleavage directed by the release agent would allow an additional degree of discrimination of target-bound from non-hybridized probes. Briefly, the 3' terminal region of the midivariant probe such as that described in Example 10 is derivatized with biotin, poly rA or other ligand. Following hybridization with target nucleic acid, the derivatized kprobe is captured on a receptor-derivatized solid support irrespective of whether it is target-associated or not. Cleavage by the ribozyme or other release agent specifically releases only those probes which are target-associated into solution. This approach has the advantage that a high affinity ligand:receptor interaction (i.e. -biotin: avidin) may be used, and that capture is directed at the entire population of probe molecules.

Detailed Description Text (101):
As will be recognized by those familiar to the art, several methods may be used to specifically label the 3' terminal region of the probe molecule. These include, but are not limited to: (1) ligating a small RNA or DNA oligonucleotide produced synthetically, containing one or more biotin ligands, to the 3' terminus with T4 RNA ligase; (2) addition of an RNA tail to the 3' terminus with E. coli poly A polymerase, using biotinylated ribonucleoside triposphates; (3) periodate oxidation of the 3' terminal residue followed by coupling to a biotinylated ligand bearing a side chain having a primary amine followed by reduction; and, (4) hybridization of a biotinylated complementary RNA to a region distal (e.g. -3' to) the target-binding region of the midivariant probe. As will also be recognized, other ligand:receptor systems may be used, although biotin:avidin is preferred for its high affinity. These systems include, but are not limited to: (a) poly rA: poly T interactions or other hybrid-forming nucleic acids (b) specific RNA binding proteins such as the coat protein of R17, and (c) high affinity antibody:antigen interactions, such as fluorescein:antifluorescein.

Detailed Description Text (104):
A hybridization reaction containing 100 fmol of the ribozyme probe from Example 10, 10 fmol of the transcription product from the KasI-restricted DNA, 100 fmol of the biotinylated cRNA, and various amounts of synthetic HIV target are combined with poly(dA)-tailed capture probes in 2.5M GTC. After 30 minutes of hybridization, the complexes are captured onto oligo dT.sub.14 -derivatized paramagnetic particles as generally described in GENE-TRAK Systems HIV assay, which assay is commercially

[redacted]

available. The particles are separated from the solution by placing the tubes in a magnetic field, the supernatants removed, and the particles washed three times with a buffer containing 1M GTC. The complexes are released by placing the particles in 2.75M GTC at 37.degree. C. for 5 minutes. The particles are removed, and the supernatant added to a suspension of streptavidin-derivatized paramagnetic particles (Advanced Magnetics, Cambridge, Mass.). The suspension is incubated 5 minutes at 37.degree. C., the particles separated from the solution in a magnetic field as above, washed three times with 200 .mu.l of 0.1M KCl, 1 mM EDTA, 0.5% NP-40, and 0.05M Tris-HCl pH7.8. After washing, the particles are suspended in 100 .mu.l of 15 mM MgCl.sub.2, 0.5% NP-40, 0.05M Tris-HCl, pH7.8 and incubated 15 minutes at 37.degree. C. The released midivariants are amplified by mixing 50 .mu.l of the supernatant from the above reaction with 50 .mu.l of a solution containing 2 .mu.g of Q.beta. replicase, 800 .mu.M each ATP, GTP, CTP, and UTP, 15 mM MgCl.sub.2, and 6.4 .mu.g/ml propidium iodide. The fluorescence displayed by the reaction is followed using a Fluoroskan instrument (Flow laboratories) or other fluorimeter capable of maintaining the reaction vessel at a constant temperature of 37.degree. . The time at which a fluorescence increase is first detected is inversely proportional to the level of HIV target RNA added to the initial hybridization.

CLAIMS:

1. A nucleic acid comprising a first section and a second section, each of said sections having a 5' and a 3' end, said nucleic acid being associated with an inhibitory element;

said first section comprising a nucleotide sequence substantially identical to MDV-1, said nucleotide sequence being capable of autocatalytic replication under reaction conditions, but incapable of autocatalytic replication while associated with said inhibitory element;

said second section being positioned at one end of said first section and being capable of assuming a bound position with a target, said second section comprising a cleavage site at which said first section is separated from said inhibitory element upon activation of a ribozyme formed by said nucleic acid and said target;

said inhibitory element being positioned at the end of said second section opposite to that of said first section;

said nucleic acid forming said ribozyme together with said target as described in the formula and structure set forth below: ##STR5## wherein the letter X generally represents said target, X.sup.1 represents a first target region comprising a nucleotide sequence which forms said ribozyme with said nucleic acid, X.sup.2 represents a terminal nucleotide of X.sup.1 or a second target region, and X.sup.3 represents a terminal nucleotide of X.sup.1 or a third target region; and the letter P generally represents said nucleic acid, P.sup.1 represents said first section, P.sup.2 represents said second section, P.sup.3 represents said inhibitory element, and P.sup.4 represents a fourth section of said nucleic acid that is capable of contributing nucleotides to form said ribozyme with X.sup.1 and P.sup.2 ;

wherein X.sup.1 and P.sup.4 are not the same sequence and are selected from the sequences 5'-MGAAAK-3'(SEQ ID NO:2) and 5'-J'CUGANGAM'-3'(SEQ ID NO:3); the letter N represents a nucleotide selected from the group consisting of A, G, U, and C; and P.sup.2 comprises the sequence 5'-K'UWJ-3' (SEQ ID NO:4) where W represents C or A, and the letters J, J', K, K', M, and M' each represent a group of four or more nucleotides, wherein J and J' are complementary to each other, K and K' are complementary to each other, and M and M' are complementary to each other.

2. The nucleic acid of claim 1 wherein said inhibitory element is capable of assuming a bound position with said target at X.sup.2.

6. The nucleic acid of claim 5, wherein said support means comprises a ligand capable of binding to an anti-ligand associated with said support.

10. A composition comprising first and second nucleic acids;

said first nucleic acid comprising a first section and a second section, each of said sections having a 5' and a 3' end, said first nucleic acid being associated with an inhibitory element;

said first section comprising a nucleotide sequence substantially identical to MDV-1, said nucleotide sequence being capable of autocatalytic replication under reaction conditions, but incapable of autocatalytic replication while associated with said inhibitory element;

said second section being positioned at one end of said first section and being capable of assuming a bound position with a target, said second section comprising a cleavage site at which said first section is separated from said inhibitory element upon activation of a ribozyme formed by said first and second nucleic acids and said target;

said inhibitory element being positioned at the end of said second section opposite to that of said first section;

said first and said second nucleic acids forming said ribozyme with said target, said ribozyme being of the formula and structure set forth below: ##STR6## wherein the letter X generally represents said target, X.sup.1 represents a first target region, X.sup.2 represents a terminal nucleotide of X.sup.1 or a second target region, and X.sup.3 represents a terminal nucleotide of X.sup.1 or a third target region; the letter P generally represents said first nucleic acid, P.sup.1 represents said first section, P.sup.2 represents said second section, P.sup.3 represents said inhibitory element, and P.sup.4 represents the terminal nucleotide of P.sup.1 ; the letter R generally represents said second nucleic acid, R.sup.1 represents a first area of said second nucleic acid that is capable of forming a ribozyme with P.sup.2 of said first nucleic acid, R.sup.2 represents a terminal nucleotide of R.sup.1 or a second area of said second nucleic acid that is capable of assuming a bound position with said target; and at least one of R.sup.1, R.sup.2, P.sup.2, and P.sup.3 is capable of assuming a bound position with said target;

wherein P.sup.2 comprises the sequence 5'-K'UWJ-3' (SEQ ID NO:49), where W is either C or U, R.sup.1 and X.sup.1 are not the same sequence and are selected from the group of sequences 5'-MGAAAK-3' (SEQ ID NO:2) and 5'-J'CUGANGAM'-3' (SEQ ID NO:3); wherein N is one of the nucleotides U, G, A, and C; and the letters J, J', K, K', M, and M' each represent a group of four or more nucleotides, wherein J and J' are complementary to each other, K and K' are complementary to each other, and M and M' are complementary to each other.

11. A composition comprising first and second nucleic acids;

said first nucleic acid comprising a first section and a second section, each of said sections having a 5' and a 3' end, and said first nucleic acid being associated with an inhibitory element;

said first section comprising a nucleotide sequence substantially identical to MDV-1, said nucleotide sequence being capable of autocatalytic replication under reaction conditions, but incapable of autocatalytic replication while associated with said inhibitory element;

said second section being positioned at one end of said first section and being capable of assuming a bound position with a target, said second section comprising a cleavage site at which said first section is separated from said inhibitory element activation of a ribozyme formed by said first and second nucleic acids in the presence of said target;

said inhibitory element being positioned at the end of said second section opposite to that of said first section;

said first and second nucleic acids forming said ribozyme with said target, said ribozyme being of the formula and structure set forth below: ##STR7## wherein the letter X generally represents said target, X.sup.1 represents a first target region, X.sup.2 represents a terminal nucleotide of X.sup.1 or a second target region, and

X.sup.3 represents a terminal nucleotide of X.sup.1 or a third target region; the letter P generally represents said first nucleic acid, P.sup.1 represents said first section, P.sup.2 represents said second section, P.sup.3 represents said inhibitory element, and P.sup.4 represents a fourth section of said first nucleic acid capable of contributing nucleotides to the formation of a ribozyme; the letter R generally represents said second nucleic acid, R.sup.1 represents a first area of said second nucleic acid that is capable of forming a ribozyme with P.sup.2, and R.sup.2 represents a terminal nucleotide of R.sup.1 or a second area of said second nucleic acid that is capable of assuming a bound position with said target; and at least one of R.sup.1, R.sup.2, P.sup.2, and P.sup.3 is capable of assuming a bound position with said target at X.sup.1 , X.sup.2, or X.sup.3 ;

wherein P.sup.2 comprises the sequence 5'-K'UWJ-3' (SEQ ID NO:49), W is either C or U, R.sup.1 and P.sup.4 are not the same sequence and are selected from the group of sequences 5'-MGAAAK-3' (SEQ ID NO:2) and 5'-J' CUGANGAM'-3' (SEQ ID NO:3); N is one of the nucleotides U, G, A, and C; and the letters J, J', K, K', M, and M' each represent a group of four or more nucleotides, wherein J and J' are complementary to each other, K and K' are complementary to each other, and M and M' are complementary to each other.

12. A composition comprising first and second nucleic acids;

said first nucleic acid comprising a first section and a second section, each of said sections having a 5' and a 3' end, said first nucleic acid being associated with an inhibitory element;

said first section comprising a nucleotide sequence substantially identical to MDV-1, said nucleotide sequence being capable of autocatalytic replication under reaction conditions, but incapable of autocatalytic replication while associated with said inhibitory element;

said second section being positioned at one end of said first section and being capable of assuming a bound position with a target, said second section comprising a cleavage site at which said first nucleic acid is separated from said inhibitory element upon activation of a ribozyme formed by said first and second nucleic acids in the presence of said target;

said inhibitory element being positioned at the end of said second section opposite to that of said first section;

said first and second nucleic acids forming said ribozyme with said target, said ribozyme being of the formula and structure set forth below: ##STR8## wherein the letter X generally represents said target, X.sup.1 represents a first target region, X.sup.2 represents a terminal nucleotide of X.sup.1 or a second target region, and X.sup.3 represents a terminal nucleotide of X.sup.1 or a third target region; the letter P generally represents said first nucleic acid, P.sup.1 represents said first section, P.sup.2 represents said second section, P.sup.3 represents said inhibitory element, and P.sup.4 represents a terminal nucleotide of P.sup.1 or a fourth section of said first nucleic acid that is capable of contributing sequences to the formation of said ribozyme; the letter R generally represents said second nucleic acid, R.sup.1 represents a first area of said second nucleic acid that is capable of forming a ribozyme with P.sup.2, and R.sup.2 represents a terminal nucleotide of R.sup.1 or a second area of said second nucleic acid that is capable of assuming a bound position with said target; and at least one of R.sup.1, R.sup.2, P.sup.2, and P.sup.3 is capable of assuming a bound position with said target at X.sup.1, X.sup.2, and X.sup.3 ;

wherein P.sup.2 comprises the sequence 5'-FNGUCQ-3' (SEQ ID NO:5), the letter N represents any one of the four nucleotides A, G, U, and C, and R.sup.1 comprises the sequence 5'-Q'AGAAF'ACCAGAGAAACACACGUUG UGGUAUAUUACCUGGUA-3' (SEQ ID NO:6); and the letters Q, Q', F, and F' each represent a group of four or more nucleotides, wherein Q and Q' are complementary to each other and F and F' are complementary to each other.

17. The composition of claim 16, wherein said support means comprises a ligand

capable of binding to an anti-ligand associated with said support.

20. A method for detecting the presence of a nucleic acid target in a sample comprising the steps of:

(a) contacting said sample with said nucleic acid of claim 1 to form a mixture;

(b) imposing binding conditions on said mixture to form a complex between said nucleic acid and said target, if present;

(c) imposing release reaction conditions and autocatalytic replication reaction conditions on said mixture to form an autocatalytic reaction product; and

(d) monitoring said mixture for the presence of said autocatalytic reaction product as a measure of the presence of said target in said sample.

21. The method of claim 20, wherein said inhibitory element is capable of assuming a bound position with said target at X.sup.2.

24. The method of claim 20, wherein said nucleic acid is associated with a support means through P.sup.3, said support means being capable of associating said nucleic acid to a support;

said method comprising the steps of binding said nucleic acid to said support through said support means, and separating substantially all unbound material from said support prior to imposing release reaction conditions and autocatalytic reaction conditions.

25. A method for detecting the presence of a nucleic acid target in a sample comprising the steps of:

(a) contacting said sample with said first and second nucleic acids of claim 10 to form a mixture;

(b) imposing binding conditions on said mixture to form a complex between said first and second nucleic acids and said target, if present;

(c) imposing release reaction conditions and autocatalytic replication reaction conditions on said mixture to form an autocatalytic reaction product; and

(d) monitoring said mixture for the presence of said autocatalytic reaction product as a measure of the presence of said target in said sample.

26. A method for detecting the presence of a nucleic acid target in a sample comprising the steps of:

(a) contacting said sample with said first and second nucleic acids of claim 11 to form a mixture;

(b) imposing binding conditions on said mixture to form a complex between said first and second nucleic acids and said target, if present;

(c) imposing release reaction conditions and autocatalytic replication reaction conditions on said mixture to form an autocatalytic reaction product; and

(d) monitoring said mixture for the presence of said autocatalytic reaction product as a measure of the presence of said target in said sample.

27. A method for detecting the presence of a nucleic acid target in a sample comprising the steps of:

(a) contacting said sample with said first and second nucleic acids of claim 12 to form a mixture;

(b) imposing binding conditions on said mixture to form a complex between said first

and second nucleic acids and said target, if present;

(c) imposing release reaction conditions and autocatalytic replication reaction conditions on said mixture to form an autocatalytic reaction product; and

(d) monitoring said mixture for the presence of said autocatalytic reaction product as a measure of the presence of said target in said sample.

31. The method of claim 25, 26, or 27, wherein said first nucleic acid is associated with a support means through P.sup.3, said support means being capable of associating said first nucleic acid to a support;

said method comprising the steps of binding said first nucleic acid to said support through said support means and separating substantially all unbound material from said support prior to imposition of release reaction conditions and autocatalytic replication conditions.

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DOCUMENT-IDENTIFIER: US 5646042 A
TITLE: C-myb targeted ribozymes

Brief Summary Text (9):
Basic fibroblast growth factor (bFGF) is another smooth muscle cell mitogen in vitro (Klagsbrun, M. and Edelman, E. R., 1989, Arteriosclerosis, 9, 269-278). In a rat model, anti-bFGF antibodies inhibit the proliferation of medial smooth muscle cells 24 to 48 hours after balloon catheter injury (Lidner, V. and Reidy, M. A., 1991, Proc. Natl. Acad. Sci. USA, 88, 3739-3743). In addition to bFGF, heparin binding epidermal growth factor (HB-EGF) (Higashiyama, S., et al., 1991, Science, 251, 936-939.), insulin-like growth factor I (IGF-I) (Banskota, N. K., et al., 1989, Molec. Endocrinol., 3, 1183-1190) and endothelin (Komuro, I., et al., 1988, FEBS Letters, 238, 249-252) have been shown to induce smooth muscle cell proliferation. A number of other factors (such as interleukin-1 and tumor necrosis factor-.alpha.) may indirectly affect smooth muscle cell proliferation by inducing the expression of PDGF (Hajjar, K. A., et al., 1987, J. Exp. Med., 166, 235-245; Raines, E. W., et al., 1989, Science, 243, 393-396).

Brief Summary Text (14):
" . . . Method is based on the delivery of proteins by catheterization to discrete blood vessel segments using genetically modified or normal cells or other vector systems . . . In addition,, catalytic RNAs, called ribozymes, can specifically degrade RNA sequences. . . . The requirements for a successful RNA cleavage include a hammerhead structure with conserved RNA sequence at the region flanking this structure . . . any GUG sequence within the RNA transcript can serve as a target for degradation by the ribozyme . . . gene transfer using vectors expressing such proteins as tPA for the treatment of thrombosis and restenosis, anglogenesis or growth factors for the purpose of revascularization . . . "

Brief Summary Text (16):
This invention relates to ribozymes, or enzymatic RNA molecules, directed to cleave mRNA species that are required for cellular growth responses. In particular, applicant describes the selection and function of ribozymes capable of cleaving RNA encoded by the oncogene, c-myb. Such ribozymes may be used to inhibit the hyper-proliferation of smooth muscle cells in restenosis and of tumor cells in numerous cancers. To block restenosis, a target molecule required for the induction of smooth muscle cell proliferation by a number of different growth factors is preferred. To this end c-myc, c-fos, and c-myb are useful targets in this invention.

Brief Summary Text (17):
Other transcription factors involved in the response to growth and proliferation signals include NF-.kappa.B, oct-1 and SRF. NF-.kappa.B protein activates cellular transcription and induces increases in cellular synthetic pathways. In a resting cell, this protein is found in the cytoplasm, complexed with its inhibitor, I-.kappa.B. Upon phosphorylation of the I-.kappa.B molecule, the complex dissociates and NF-.kappa.B is released for transport to the nucleus, where it binds DNA and induces transcriptional activity in (NF-.kappa.B)-responsive genes. One of the (NF-.kappa.B)-responsive genes is the NF-.kappa.B gene itself. Thus, release of the NF-.kappa.B protein from the inhibitory complex results in a cascade of gene expression which is auto-induced. Early inhibition of NF-.kappa.B can reduce expression of a number of genes required for growth and proliferation, such as

c-myb.

Brief Summary Text (19):
While some of the above mentioned studies demonstrated that antisense oligonucleotides can efficiently reduce the expression of factors required for smooth muscle cell proliferation, enzymatic RNAs, or ribozymes have yet to be demonstrated to inhibit smooth muscle cell proliferation. Such ribozymes, with their catalytic activity and increased site specificity (as described below), represent more potent and safe therapeutic molecules than antisense oligonucleotides. In the present invention, ribozymes that cleave c-myb mRNA are described. Moreover, applicant shows that these ribozymes are able to inhibit smooth muscle cell proliferation and that the catalytic activity of the ribozymes is required for their inhibitory effect. From those of ordinary skill in the art, it is clear from the examples described, that other ribozymes that cleave target mRNAs required for smooth muscle cell proliferation may be readily designed and are within the invention.

Brief Summary Text (20):
By "inhibit" is meant that the activity of c-myb or level of mRNAs encoded by c-myb is reduced below that observed in the absence of the nucleic acid, particularly, inhibition with ribozymes and preferably is below that level observed in the presence of an inactive RNA molecule able to bind to the same site on the mRNA, but unable to cleave that RNA.

Brief Summary Text (21):
By "enzymatic nucleic acid molecule" it is meant a nucleic acid molecule which has complementarity in a substrate binding region to a specified gene target, and also has an enzymatic activity which is active to specifically cleave RNA in that target. That is, the enzymatic nucleic acid molecule is able to intermolecularly cleave RNA and thereby inactivate a target RNA molecule. This complementarity functions to allow sufficient hybridization of the enzymatic nucleic acid molecule to the target RNA to allow the cleavage to occur. One hundred percent complementarity is preferred, but complementarity as low as 50-75% may also be useful in this invention. By "equivalent" RNA to c-myb is meant to include those naturally occurring RNA molecules associated with restenosis and cancer in various animals, including human, rat and pig. Such a molecule will generally contain some ribonucleotides, but the other nucleotides may be substituted at the 2'-hydroxyl position and in other locations with other moeities as discussed below.

Brief Summary Text (23):
Six basic varieties of naturally-occurring enzymatic RNAs are known presently. Each can catalyze the hydrolysis of RNA phosphodiester bonds in trans (and thus can cleave other RNA molecules) under physiological conditions. Table I summarizes some of the characteristics of these ribozymes. In general, enzymatic nucleic acids act by first binding to a target RNA. Such binding occurs through the target binding portion of a enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through complementary base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a target RNA will destroy its ability to direct synthesis of an encoded protein. After an enzymatic nucleic acid has bound and cleaved its RNA target, it is released from that RNA to search for another target and can repeatedly bind and cleave new targets.

Brief Summary Text (24):
The enzymatic nature of a ribozyme is advantageous over other technologies, such as antisense technology (where a nucleic acid molecule simply binds to a nucleic acid target to block its translation) since the concentration of ribozyme necessary to affect a therapeutic treatment is lower than that of an antisense oligonucleotide. This advantage reflects the ability of the ribozyme to act enzymatically. Thus, a single ribozyme molecule is able to cleave many molecules of target RNA. In addition, the ribozyme is a highly specific inhibitor, with the specificity of inhibition depending not only on the base pairing mechanism of binding to the target RNA, but also on the mechanism of target RNA cleavage. Single mismatches, or base-substitutions, near the site of cleavage can completely eliminate catalytic

activity of a ribozyme. Similar mismatches in antisense molecules do not prevent their action (Woolf, T. M., et al., 1992, Proc. Natl. Acad. Sci. USA, 89, 7305-7309). Thus, the specificity of action of a ribozyme is greater than that of an antisense oligonucleotide binding the same RNA site.

Brief Summary Text (25):
In preferred embodiments of this invention, the enzymatic nucleic acid molecule is formed in a hammerhead or hairpin motif, but may also be formed in the motif of a hepatitis delta virus, group I intron or RNaseP RNA (in association with an RNA guide sequence) or Neurospora VS RNA. Examples of such hammerhead motifs are described by Rossi et al., 1992, Aids Research and Human Retroviruses 8, 183, of hairpin motifs by Hampel et al., EP0360257, Hampel and Tritz, 1989 Biochemistry 28, 4929, and Hampel et al., 1990 Nucleic Acids Res. 18, 299, and an example of the hepatitis delta virus motif is described by Perrotta and Been, 1992 Biochemistry 31, 16; of the RNaseP motif by Guerrier-Takada et al., 1983 Cell 35, 849, Neurospora VS RNA ribozyme motif is described by Collins (Saville and Collins, 1990 Cell 61, 685-696; Saville and Collins, 1991 Proc. Natl. Acad. Sci. USA 88, 8826-8830; Collins and Olive, 1993 Biochemistry 32, 2795-2799) and of the Group I intron by Cech et al., U.S. Pat. No. 4,987,071. These specific motifs are not limiting in the invention and those skilled in the art will recognize that all that is important in an enzymatic nucleic acid molecule of this invention is that it has a specific substrate binding site which is complementary to one or more of the target gene RNA regions, and that it have nucleotide sequences within or surrounding that substrate binding site which impart an RNA cleaving activity to the molecule.

Brief Summary Text (26):
In a preferred embodiment the invention provides a method for producing a class of enzymatic cleaving agents which exhibit a high degree of specificity for the RNA of a desired target. The enzymatic nucleic acid molecule is preferably targeted to a highly conserved sequence region of a target mRNAs encoding c-myb proteins such that specific treatment of a disease or condition can be provided with either one or several enzymatic nucleic acids. Such enzymatic nucleic acid molecules can be delivered exogenously to specific cells as required. Alternatively, the ribozymes can be expressed from DNA/RNA vectors that are delivered to specific cells.

Brief Summary Text (27):
Synthesis of nucleic acids greater than 100 nucleotides in length is difficult using automated methods, and the therapeutic cost of such molecules is prohibitive. In this invention, small enzymatic nucleic acid motifs (e.g., of the hammerhead or the hairpin structure) are used for exogenous delivery. The simple structure of these molecules increases the ability of the enzymatic nucleic acid to invade targeted regions of the mRNA structure. However, these catalytic RNA molecules can also be expressed within cells from eukaryotic promoters (e.g., Scanlon et al., 1991, Proc. Natl. Acad. Sci. USA, 88, 10591-5; Kashani-Sabet et al., 1992 Antisense Res. Dev., 2, 3-15; Dropulic et al., 1992 J. Virol, 66, 1432-41; Weerasinghe et al., 1991 J. Virol, 65, 5531-4; Ojwang et al., 1992 Proc. Natl. Acad. Sci. USA 89, 10802-6; Chen et al., 1992 Nucleic Acids Res., 20, 4581-9; Sarver et al., 1990 Science 247, 1222-1225). Those skilled in the art realize that any ribozyme can be expressed in eukaryotic cells from the appropriate DNA/RNA vector. The activity of such ribozymes can be augmented by their release from the primary transcript by a second ribozyme (Draper et al., PCT WO93/23569, and Sullivan et al., PCT WO94/02595, both hereby incorporated in their totality by reference herein; Ohkawa et al., 1992 Nucleic Acids Symp. Ser., 27, 15-6; Taira et al., 1991, Nucleic Acids Res., 19, 5125-30; Ventura et al., 1993 Nucleic Acids Res., 21, 3249-55; Chowrira et al., 1994 J. Biol. Chem. 269, 25656).

Brief Summary Text (28):
Thus, in a first aspect, the invention features ribozymes that inhibit cell proliferation. These chemically or enzymatically synthesized RNA molecules contain substrate binding domains that bind to accessible regions of their target mRNAs. The RNA molecules also contain domains that catalyze the cleavage of RNA. The RNA molecules are preferably ribozymes of the hammerhead or hairpin motif. Upon binding, the ribozymes cleave the target mRNAs, preventing translation and protein accumulation. In the absence of the expression of the target gene, cell proliferation is inhibited.

Brief Summary Text (31):
Ribozymes are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells. The nucleic acid or nucleic acid complexes can be locally administered to relevant tissues ex vivo, or in vivo through injection, infusion pump or stent, with or without their incorporation in biopolymers.

Brief Summary Text (32):
In another aspect of the invention, ribozymes that cleave target molecules and inhibit c-myb activity are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors are preferably DNA plasmids or viral vectors. Ribozyme expressing viral vectors could be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. Preferably, the recombinant vectors capable of expressing the ribozymes are delivered as described above, and persist in target cells. Alternatively, viral vectors may be used that provide for transient expression of ribozymes. Such vectors might be repeatedly administered as necessary. Once expressed, the ribozymes cleave the target mRNA. Delivery of ribozyme expressing vectors could be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that would allow for introduction into the desired target cell.

Brief Summary Text (33):
By "vectors" is meant any nucleic acid- and/or viral-based technique used to deliver a desired nucleic acid.

Brief Summary Text (34):
In preferred embodiments, the ribozymes have binding arms which are complementary to the sequences in the tables, shown as Seq. I.D. Nos. 1-100. Examples of such ribozymes are shown as Seq. I.D. Nos. 101-129. Those in the art will recognize that while such examples are designed to mouse RNA, similar ribozymes can be made complementary to human RNA. By complementary is thus meant that the binding arms are able to cause cleavage of a human or mouse mRNA target. Examples of such ribozymes consist essentially of sequences defined as Seq. I.D. Nos. 101-129 below. By "consists essentially of" is meant that the active ribozyme contains an enzymatic center equivalent to those in the examples, and binding arms able to bind human mRNA such that cleavage at the target site occurs. Other sequences may be present which do not interfere with such cleavage.

Brief Summary Text (35):
In another aspect of the invention, ribozymes that cleave target molecules and inhibit cell proliferation are expressed from transcription units inserted into DNA, RNA, or vital vectors. Preferably, the recombinant vectors capable of expressing the ribozymes are locally delivered as described above, and transiently persist in smooth muscle cells. Once expressed, the ribozymes cleave their target mRNAs and prevent proliferation of their host cells. The recombinant vectors are preferably DNA plasmids or adenovirus vectors. However, other mammalian cell vectors that direct the expression of RNA may be used for this purpose.

Drawing Description Text (4):
FIG. 1 is a diagrammatic representation of the hammerhead ribozyme domain known in the art. Stem II can be .gtoreq.2 base-pair long.

Drawing Description Text (5):
FIG. 2a is a diagrammatic representation of the hammerhead ribozyme domain known in the art; FIG. 2b is a diagrammatic representation of the hammerhead ribozyme as divided by Uhlenbeck (1987, Nature, 327, 596-600) into a substrate and enzyme portion; FIG. 2c is a similar diagram showing the hammerhead divided by Haseloff and Gerlach (1988, Nature, 334, 585-591) into two portions; and FIG. 2d is a similar diagram showing the hammerhead divided by Jeffries and Symons (1989, Nucl. Acids. Res., 17, 1371-1371) into two portions.

Drawing Description Text (6):
FIG. 3 is a diagrammatic representation of the general structure of a hairpin

ribozyme. Helix 2 (H2) is provided with a least 4 base pairs (i.e., n is 1, 2, 3 or 4) and helix 5 can be optionally provided of length 2 or more bases (preferably 3-20 bases, i.e., m is from 1-20 or more). Helix 2 and helix 5 may be covalently linked by one or more bases (i.e., r is .gtoreq.1 base). Helix 1, 4 or 5 may also be extended by 2 or more base pairs (e.g., 4-20 base pairs) to stabilize the ribozyme structure, and preferably is a protein binding site. In each instance, each N and N' independently is any normal or modified base and each dash represents a potential base-pairing interaction. These nucleotides may be modified at the sugar, base or phosphate. Complete base-pairing is not required in the helices, but is preferred. Helix 1 and 4 can be of any size (i.e., o and p is each independently from 0 to any number, e.g., 20) as long as some base-pairing is maintained. Essential bases are shown as specific bases in the structure, but those in the art will recognize that one or more may be modified chemically (abasic, base, sugar and/or phosphate modifications) or replaced with another base without significant effect. Helix 4 can be formed from two separate molecules, i.e., without a connecting loop. The connecting loop when present may be a ribonucleotide with or without modifications to its base, sugar or phosphate. "q" is .gtoreq.2 bases. The connecting loop can also be replaced with a non-nucleotide linker molecule. H refers to bases A, U, or C. Y refers to pyrimidine bases. "--" refers to a covalent bond.

Drawing Description Text (9):
FIG. 6 is a schematic representation of an RNAseH accessibility assay. Specifically, the left side of FIG. 6 is a diagram of complementary DNA oligonucleotides bound to accessible sites on the target RNA. Complementary DNA oligonucleotides are represented by broad lines labeled A, B, and C. Target RNA is represented by the thin, twisted line. The right side of FIG. 6 is a schematic of a gel separation of uncut target RNA from a cleaved target RNA. Detection of target RNA is by autoradiography of body-labeled, T7 transcript. The bands common to each lane represent uncleaved target RNA; the bands unique to each lane represent the cleaved products.

Drawing Description Text (10):
FIG. 7 is a graph of the results of an RNAseH accessibility assay of murine c-myb RNA. On the abscissa is the sequence number of the DNA oligonucleotide that is homologous to the ribozyme target site. The ordinate represents the percentage of the intact transcript that was cleaved by RNAse H.

Drawing Description Text (12):
FIG. 9 shows the effect of chemical modifications on the catalytic activity of hammerhead ribozyme targeted to c-myb site 575. A) diagrammatic representation of 575 hammerhead ribozyme.cndot.substrate complex. 2'-O-methyl ribozyme represents a hammerhead (HH) ribozyme containing 2'-O-methyl substitutions at five nucleotides in the 5' and 3' termini. 2'-O-methyl P=S ribozyme represents a hammerhead (HH) ribozyme containing 2'-O-methyl and phosphorothioate substitutions at five nucleotides in the 5' and 3' termini. 2'-C-allyl iT ribozyme represents a hammerhead containing ribose residues at five positions. The remaining 31 nucleotide positions contain 2'-hydroxyl group substitutions, wherein 30 nucleotides contain 2'-O-methyl substitutions and one nucleotide (U.sub.4) contains 2'-C-allyl substitution. Additionally, 3' end of this ribozyme contains a 3'-3' linked inverted T. 2'-C-allyl P=S ribozyme is similar to 2'-C-allyl iT ribozyme with the following changes: five nucleotides at the 5' and 3' termini contain phosphorothioate substitutions and the ribozyme lacks the 3'-end inverted T modification. B) shows the ability of ribozymes described in FIG. 9A to inhibit smooth muscle cell proliferation.

Drawing Description Text (16):
FIG. 13 shows the effect of phosphorthioate substitutions on the catalytic activity of 2'-C-allyl 575 HH ribozyme. A) diagrammatic representation of 575 hammerhead ribozyme.cndot.substrate complex. 10 P=S 5' and 3' ribozyme is identical to the 2'-C-allyl P=S ribozyme described in FIG. 9. 5 P=S 3' ribozyme is same as 10 P=S 5' and 3' ribozyme, with the exception that only five nucleotides at the 3' termini contain phosphorothioate substitutions. 5 P=S Loop ribozyme is similar to 2'-C-allyl iT described in FIG. 9, with the exception that five nucleotides within loop II of this ribozyme contain phosphorothioate substitutions. 5 P=S 5' ribozyme is same as 10 P=S 5' and 3' ribozyme, with the exception that only five nucleotides at the 5' termini contain phosphorothioate substitutions. Additionally, this ribozyme contains

a 3'-3' linked inverted T at its 3' end. B) shows the ability of ribozymes described in FIG. 13A to inhibit smooth muscle cell proliferation.

Drawing Description Text (18):
FIG. 15 shows the effect of varying the length of substrate binding arm of 575 HH ribozyme on the inhibition of smooth muscle cell proliferation.

Detailed Description Text (1):
Target sites

Detailed Description Text (2):
Targets for useful ribozymes can be determined as disclosed in Draper et al supra, Sullivan et al., supra, as well as by Draper et al., "Method and reagent for treatment of arthritic conditions U.S. Ser. No. 08/152,487, filed Nov. 12, 1993, and hereby incorporated by reference herein in totality. Rather than repeat the guidance provided in those documents here, below are provided specific examples of such methods, not limiting to those in the art. Ribozymes to such targets are designed as described in those applications and synthesized to be tested in vitro and in vivo, as also described. Such ribozymes can also be optimized and delivered as described therein. While specific examples to mouse RNA are provided, those in the art will recognize that equivalent human RNA targets can be used as described below. Thus, the same target may be used, but binding arms suitable for targetting human RNA sequences are present in the ribozyme. Such targets may also be selected as described below.

Detailed Description Text (3):
The sequence of human, pig and murine c-myb mRNAs were screened for optimal ribozyme target sites using a computer folding algorithm. Hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables II and IV (All sequences are 5' to 3' in the tables) The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme. While murine, pig and human sequences can be screened and ribozymes thereafter designed, the human targeted sequences are of most utility. However, as discussed in Stinchcomb et al., "Method and Composition for Treatment of Restenosis and Cancer Using Ribozymes," filed May 18, 1994, U.S. Ser. No. 08/245,466, murine and pig targeted ribozymes may be useful to test efficacy of action of the ribozyme prior to testing in humans. The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme.

Detailed Description Text (4):
Hammerhead or hairpin ribozymes were designed that could bind and were individually analyzed by computer folding (Jaeger et al., 1989 Proc. Natl. Acad. Sci. USA, 86, 7706) to assess whether the ribozyme sequences fold into the appropriate secondary structure. Those ribozymes with unfavorable intramolecular interactions between the binding arms and the catalytic core are eliminated from consideration. Varying binding arm lengths can be chosen to optimize activity. Generally, at least 5 bases on each arm are able to bind to, or otherwise interact with, the target RNA.

Detailed Description Text (5):
The sequences of the ribozymes that are chemically synthesized, useful in this study, are shown in Table III. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. For example, stem-loop II sequence of hammerhead ribozymes listed in Table III (5'-GGCCGAAAGGCC-3') can be altered (substitution, deletion, and/or insertion) to contain any sequences provided a minimum of two base-paired stem structure can form. Similarly, stem-loop IV sequence of hairpin ribozymes listed in Table III (5'-CACGUUGUG-3') can be altered (substitution, deletion, and/or insertion) to contain any sequence, provided a minimum of two base-paired stem structure can form. The sequences listed in Table III may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

Detailed Description Text (7):
Ribozyme activity can be optimized as described in this application. These include

altering the length of the ribozyme binding arms (stems I and III, see FIG. 2c), or chemically synthesizing ribozymes with modifications that prevent their degradation by serum ribonucleases (see e.g., Eckstein et al., International Publication No. WO 92/07065; Perrault et al., 1990 Nature 344, 565; Pieken et al., 1991 Science 253, 314; Usman and Cedergren, 1992 Trends in Biochem. Sci. 17, 334; Usman et al., International Publication No. WO 93/15187; and Rossi et al., International Publication No. WO 91/03162, as well as Usman, N. et al. U.S. patent application Ser. No. 07/829,729, and Sproat, European Patent Application 92110298.4 which describe various chemical modifications that can be made to the sugar moieties of enzymatic RNA molecules, modifications which enhance their efficacy in cells, and removal of stem II bases to shorten RNA synthesis times and reduce chemical requirements. (All these publications are hereby incorporated by reference herein.),

Detailed Description Text (14):
The following examples demonstrate the selection of ribozymes that cleave c-myb mRNA. The methods described herein represent a scheme by which ribozymes may be derived that cleave other mRNA targets required for cell division. Also provided is a description of how such ribozymes may be delivered to smooth muscle cells. The examples demonstrate that upon delivery, the ribozymes inhibit cell proliferation in culture. Moreover, no inhibition is observed if mutated ribozymes that are catalytically inactive are applied to the cells. Thus, inhibition requires the catalytic activity of the ribozymes. The cell division assay used represents a model system for smooth muscle cell hyperproliferation in restenotic lesions.

Detailed Description Text (17):
The sequence of human c-myb mRNA was screened for accessible sites using a computer folding algorithm. Regions of the mRNA that did not form secondary folding structures and contained potential hammerhead ribozyme cleavage sites were identified. These sites are shown in Table II and are identical to Table I of Draper, "Method and Reagent for Treatment of a Stenotic Condition", U.S. Ser. No. 07/987,132. (All sequences are 5' to 3' in the tables.) In the original, the sites were identified using nucleotide numbers from (Majello, B., et al., 1986, Proc. Natl. Acad. Sci. USA, 83, 9636-9640) (GenBank Accession No. M15024). Here, we report sites using the sequence numbers from (Westin, E. H., et al., 1990, Oncogene, 5, 1117-1124) (GenBank Accession No. X52125); the latter sequence is derived from a longer c-myb cDNA isolate and thus is more representative of the full-length RNA.

Detailed Description Text (20):
To test whether the sites predicted by the computer-based RNA folding algorithm corresponded to accessible sites in c-myb RNA, 41 hammerhead sites were selected for analysis. Ribozyme target sites were chosen by comparing cDNA sequences of mouse and human c-myb (GenBank Accession No. X02774 and GenBank Accession No. X52125, repsectively) and prioritizing the sites on the basis of overall nucleotide sequence homology. Hammerhead ribozymes were designed that could bind each target (see FIG. 2C) and were individually analyzed by computer folding (Jaeger, J. A., et al., 1989, Proc. Natl. Acad. Sci USA, 86, 7706-7710) to assess whether the ribozyme sequences fold into the appropriate secondary structure. Those ribozymes with unfavorable intramolecular interactions between the binding arms and the catalytic core were eliminated from consideration. As noted below, varying binding arm lengths can be chosen to optimize activity. Generally, at least 5 bases on each arm are able to bind to, or otherwise interact with, the target RNA.

Detailed Description Text (23):
Murine and human mRNA was screened for accessible cleavage sites by the method described generally in McSwiggen, U.S. patent application No. 07/883,849 filed May 1, 1992, entitled "Assay for ribozyme target site," hereby incorporated by reference herein. Briefly, DNA oligonucleotides representing 41 potential hammerhead ribozyme cleavage sites were synthesized. A polymerase chain reaction was used to generate a substrate for T7 RNA polymerase transcription from human or murine c-myb cDNA clones. Labeled RNA transcripts were synthesized in vitro from the two templates. The oligonucleotides and the labeled transcripts were annealed, RNAseH was added and the mixtures were incubated for the designated times at 37.degree. C. Reactions were stopped and RNA separated on sequencing polyacrylamide gels. The percentage of the substrate cleaved was determined by autoradiographic quantitation using a phosphor

imaging system. The results are shown in FIGS. 7 and 8. From these data, 20 hammerhead ribozyme sites were chosen as the most accessible (see Table III). Eighteen of the twenty sites chosen overlap sequences shown in Table II; thus, the RNA folding is predictive of accessible regions in the RNA.

Detailed Description Text (26):
Ribozymes of the hammerhead or hairpin motif were designed to anneal to various sites in the mRNA message. The binding arms are complementary to the target site sequences described above. The ribozymes were chemically synthesized. The method of synthesis used followed the procedure for normal RNA synthesis as described in Usman et al., 1987 J. Am. Chem. Soc., 109, 7845 and in Scaringe et al., 1990 Nucleic Acids Res., 18, 5433 and made use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The average stepwise coupling yields were >98%. Inactive ribozymes were synthesized by substituting a U for G.sub.5 and a U for A.sub.14 (numbering from Hertel et al., 1992 Nucleic Acids Res., 20, 3252). Hairpin ribozymes were synthesized in two parts and annealed to reconstruct the active ribozyme (Chowrira and Burke, 1992 Nucleic Acids Res., 20, 2835-2840). Ribozymes were also synthesized from DNA templates using bacteriophage T7 RNA polymerase (Milligan and Uhlenbeok, 1989, Methods Enzymol. 180, 51). All ribozymes were modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-flouro, 2'-O-methyl, 2'-H (for a review see Usman and Cedergren, 1992 TIBS 17, 34). Ribozymes were purified by gel electrophoresis using general methods or were purified by high pressure liquid chromatography (HPLC; See Usman et al., Synthesis, deprotection, analysis and purification of RNA and ribozymes, filed May, 18, 1994, U.S. Ser. No. 08/245,736 the totality of which is hereby incorporated herein by reference) and were resuspended in water. The sequences of the chemically synthesized ribozymes used in this study are shown below in Table III.

Detailed Description Text (28):
Ribozyme Cleavage of Long Substrate RNA Corresponding to c-myb mRNA Target

Detailed Description Text (29):
Hammerhead-type ribozymes which were targeted to the murine c-myb mRNA were designed and synthesized to test the cleavage activity at the 20 most accessible sites in in vitro transcripts of both mouse and human c-myb RNAs. The target sequences and the nucleotide location within the c-myb mRNA are given in Table IV. All hammerhead ribozymes were synthesized with binding arm (Stems I and III; see FIG. 2C) lengths of seven nucleotides. Two hairpin ribozymes were synthesized to sites 1632 and 2231. The relative abilities of these ribozymes to cleave both murine and human RNAs is summarized in Table IV. Ribozymes (1 .mu.M) were incubated with .sup.32 P-labeled substrate RNA (prepared as described in Example 3, approximately 20 nM) for 60 minutes at 37.degree. C. using buffers described previously. Intact RNA and cleavage products were separated by electrophoresis through polyacrylamide gels. The percentage of cleavage was determined by Phosphor Imager.RTM. quantitation of bands representing the intact substrate and the cleavage products.

Detailed Description Text (30):
Five hammerhead ribozymes (directed against sites 549, 575, 1553, 1597, and 1635) and one hairpin ribozyme (directed against site 1632) were very active; they cleaved >70% of both murine and human c-myb RNA in 60 minutes. Nine of the hammerhead ribozymes (directed against sites 551, 634, 936, 1082, 1597, 1721, 1724, 1895, and 1943) were intermediate in activity, cleaving >50% of both murine and human c-myb RNA in 60 minutes. All of the sites cleaved by these active ribozymes were predicted to be accessible to ribozyme cleavage in Table 2. Six hammerhead ribozymes and one hairpin ribozyme showed low activity on at least one of the substrates. The observed differences in accessibility between the two species of c-myb RNA demonstrate the sensitivity of ribozyme action to RNA structure and suggest that even when homologous target sequences exist, ribozymes may be excluded from cleaving that RNA by structural constraints. This level of specificity minimizes non-specific toxicity of ribozymes within cells.

Detailed Description Text (32):
Ability of Hammerhead Ribozymes to Inhibit Smooth Muscle Cell Proliferation

Detailed Description Text (36):
Six hammerhead ribozymes, including the best five ribozymes from the in vitro RNA cleavage test (directed against sites 549, 575, 1553, 1598, and 1635) and one with intermediate cleavage levels (directed against site 1597) and their catalytically inactive controls were synthesized and purified as described above. The ribozymes were delivered at a concentration of 0.3 .mu.M, complexed with DMRIE/DOPE such that the cationic lipid charges and the anionic RNA charges were at 1:1 molar ratio. The results, shown in Table V, demonstrate a considerable range in the efficacy of ribozymes directed against different sites. Five of the six hammerhead ribozymes (directed against sites 549, 575, 1553, 1597, and 1598) significantly inhibit smooth muscle cell proliferation. The control, inactive ribozymes that cannot cleave c-myb RNA due to alterations in their catalytic core sequence fail to inhibit rat smooth muscle cell proliferation. Thus, inhibition of cell proliferation by these five hammerhead sequences is due to their ability to cleave c-myb RNA, and not because of any antisense activity. The sixth ribozyme (directed against site 1635) fails to function in smooth muscle cells. This ribozyme cleaved c-myb RNA very efficiently in vitro. In this experiment, 10% FBS (no ribozyme added) induced 64.+-.1% proliferation; 0% FBS produced a background of 9.+-.1% proliferation.

Detailed Description Text (39):
In addition to the hammerhead ribozymes tested above, a bipartite hairpin ribozyme (Chowrira, B. M., supra, 1992, Nucleic Acids Res., 20, 2835-2840) was identified that also cleaves c-myb RNA. The effect of this ribozyme on smooth muscle cell proliferation was tested. Ribozymes were delivered at the indicated doses with Lipofectamine at a 1:1 charge ratio. In this experiment, 10% FBS (no ribozyme) induced 87.+-.1% proliferation; 0% FBS produced 5.+-.1% proliferation. The results of a dose-response experiment are shown in Table VI. In this example, the control was an irrelevant hammerhead ribozyme. The irrelevant ribozyme control contains the same catalytic core sequences, but has binding arms that are directed to a cellular RNA that is not required for smooth muscle cell proliferation. This control failed to significantly inhibit cell proliferation, demonstrating the sequence specificity of these ribozymes. Another control that could be run is an irrelevant catalytically active ribozyme having the same GC content as the test ribozyme.

Detailed Description Text (42):
If the inhibition of proliferation observed in Example 6 is caused by the ribozymes, the level of inhibition should be proportional to the dose of RNA added. Rat aortic smooth muscle cells were assayed for proliferation in the presence of differing doses of two hammerhead ribozymes. The results shown in Table VII indicate that two hammerhead ribozymes that cleave c-myb RNA at sites 575 and 549 inhibit SMC proliferation in a dose-dependent fashion. Ribozymes were delivered with the cationic lipid, Lipofectamine at a 1:1 charge ratio. In this experiment, 10% FBS (no ribozyme) gave 92.+-.1% proliferation; 0% FBS gave 6.+-.1% proliferation. The control is an active ribozyme directed against an irrelevant mRNA target and shows no inhibition over the dose range tested. The control ribozyme contains the same catalytic core sequences as the active ribozymes but differs in its binding arm sequences (stems I and III in FIG. 2c). Thus, ribozyme inhibition of smooth muscle cell proliferation requires sequence-specific binding by the hammerhead arms to c-myb mRNA.

Detailed Description Text (45):
The experiment in Table VIII shows the response of rat smooth muscle cells to a hammerhead ribozyme that cleaves c-myb RNA at site 575 delivered with two different cationic lipids, DMRIE and Lipofectamine. Similar efficacy is observed with either lipid. 10% FBS (no ribozyme) induced 78.+-.2% proliferation; 0% FBS produced a background of 6.+-.1% proliferation.

Detailed Description Text (48):
The exact configuration of each ribozyme can be optimized by altering the length of the binding arms (stems I and III, see FIG. 2C). The length of the binding arms may have an effect on both the binding and the catalytic cleavage step (Herschlag, D., 1991, Proc. Natl. Acad. Sci. U S A, 88, 6921-5). For example, Table IX shows the ability of arm length variants of c-myb hammerhead 575 to inhibit SMC proliferation. Note that the dose used in this experiment (0.1 .mu.M) is 3-fold lower than in previous experiments. At this concentration, the 7/7 arm variant gives relatively

little inhibition. In this case, the degree of inhibition increases with concomitant increases in arm length.

Detailed Description Text (49):
The optimum arm length may be site-specific and should be determined empirically for each ribozyme. Towards this end, hammerhead ribozymes target with 7 nucleotide binding arms (7/7) and ribozymes with 12 nucleotide binding arms (12/12) targeted to three different cleavage sites were compared.

Detailed Description Text (50):
Ribozymes were delivered at 0.2 .mu.M with the cationic lipid DMRIE at a 1:1 charge ratio of oligonucleotide to cationic lipid as described in Example 6. The data are shown below in Table X. As can be seen, all three ribozymes demonstrated enhanced inhibition of smooth muscle cell proliferation with twelve nucleotide binding arms. Each ribozyme showed greater inhibition than its catalytically inactive control, again demonstrating that the ribozymes function via their ability to cleave c-myb RNA. In this experiment, 10% stimulation resulted in 54.+-.2% cell proliferation; unstimulated cells showed 8.+-.0.5% cell proliferation.

Detailed Description Text (53):
A number of substances that effect the trafficking of macromolecules through the endosome have been shown to enhance the efficacy of DNA delivery to cells. These include, but are not limited to, ammonium chloride, carbonyl cyanide p-trifluoromethoxy phenyl hydrazone (FCCP), chloroquine, monensin, colchicine, and viral particles (Cotten, M. et al., 1990, Proc. Natl. Acad. Sci. USA, 87, 4033-4037; Cotten, M. et al., 1993, J. Virol., 67, 3777-3785; Cotten, M. et al., 1992, Proc. Natl. Acad. Sci USA, 89, 6094-6098; Cristiano, R. J. et al., 1993, Proc. Natl. Acad. Sci. U S A, 90, 2122-6; Cudel, D. T. et al., 1991, Proc. Nat. Acad. Sci., USA, 88, 8850-8854; Ege, T. et al., 1984, Exp. Cell Res., 155, 9-16; Harris, C. E. et al., 1993, Am. J. Respir. Cell Mol. Biol., 9, 441-7; Seth, P. et al., 1994, J. Virol., 68, 933-40; Zenke, M. et al., 1990, Proc. Natl. Acad. Sci. USA, 87, 3655-3659). It is thought that DNA is taken up by cells by endocytosis, resulting in DNA accumulation in endosomes (Akhtar, S. and Juliano, R. L., 1992, Trends Cell Biol., 2, 139-144). Thus, the above agents may enhance DNA expression by promoting DNA release from endosomes. To determine whether such agents may augment the functional delivery of RNA and ribozymes to smooth muscle cells, the effects of chloroquine on ribozyme inhibition of smooth muscle cell proliferation were assessed. A ribozyme with twelve nucleotide binding arms that cleaves c-ruby RNA was delivered to rat smooth muscle cells as described in Example 6 (0.2 .mu.M ribozyme complexed with DMRIE/DOPE at a 1:1 charge ratio). In some cases, 10 .mu.M chloroquine was added upon stimulation of the cells. The addition of choloroquine had no effect on untreated cells (stimulation with 10% serum in the presence or absence of chloroquine resulted in 80.5.+-.1.5% and 83.+-.2% cell proliferation, respectively; unstimulated cells with and without chloroquine showed 7.+-.0.5% and 7.+-.1% cell proliferation, respectively). As shown in Table XI below, addition of chloroquine augments ribozyme inhibition of smooth muscle cell proliferation two- to three-fold.

Detailed Description Text (55):
Effect of a Hammerhead Ribozyme on Human Smooth Muscle Cell Proliferation

Detailed Description Text (56):
The hammerhead ribozyme that cleaves human c-myb RNA at site 549 was tested for its ability to inhibit human aortic smooth muscle cell proliferation. The binding site for this ribozyme is completely conserved between the mouse and human cDNA sequences. Human aortic smooth muscle cells (AOSMC) were obtained from Clonetics and were grown in SmGM (Clonetics.RTM.). Cells from passage five or six were used for assays. Conditions for the proliferation assay were the same as for the rat cells (see Example 6), except that the cells were plated in SmGM and starved in SmBM plus 0.5% FBS. The ribozyme that cleaves site 549 was delivered at varying doses complexed with the cationic lipid DMRIE at a 1:1 charge ratio. In this experiment, 10% FBS (no ribozyme) induced 57.+-.7% proliferation; the uninduced background was 6.+-.1% proliferation. The results in Table XII show that inhibition is observed over a similar concentration range as was seen with rat smooth muscle cells.

Detailed Description Text (59):
A hammerhead ribozyme that cleaves site 575 was chemically synthesized with 12 nucleotide binding arms (sequence ID NO. 127, in Table III). Chemically modified nucleotides were incorporated into this ribozyme that have been shown to enhance ribozyme stability in serum without greatly impacting catalytic activity. (See Eckstein et al., International Publication No. WO 92/07065, Perrault et al., 1990, Nature, 344, 565-568, Pieken, W. et al. 1991, Science, 253, 314-317, Usman, N.; Cedergren, R. J., 1992, Trends in Biochem. Sci., 17, 334-339, Usman, N. et al. U.S. patent application Ser. No. 07/829,729, and Sproat, B. European Patent Application 92110298.4 describe various chemical modifications that can be made to the sugar moieties of enzymatic RNA molecules. All these publications are hereby incorporated by reference herein.) The modifications used were as follows. All the nucleotides of the ribozyme contained 2'-O-methyl groups with the following exceptions: U.sub.4 and U.sub.7 contained 2'-amino substitutions; G.sub.5, A.sub.6, G.sub.8, G.sub.12, and A.sub.15.1 were 2'-OH ribonucleotides (numbering as in FIG. 1). An inactive ribozyme was chemically synthesized in which G.sub.5 and A.sub.14 were substituted with 2'-O-methyl U. Ribozymes were added to rat smooth muscle cells at the indicated concentrations as per Example 6 except that cationic lipids were omitted. Proliferation was assessed by BrdU incorporation and staining. Table XIII shows that the modified ribozyme is capable of inhibiting rat smooth muscle cell proliferation without addition of cationic lipids. In this experiment, 10% serum induced 45.+-.2% proliferation while uninduced cells showed a background of 2.3.+-.0.1% proliferation.

Detailed Description Text (62):
Tables IX and X demonstrate one means of optimizing ribozyme activity. By altering the length of the ribozyme binding arms (stems I and Ill, see FIG. 2c), the ability of the ribozyme to inhibit smooth muscle cell proliferation is greatly enhanced. Ribozymes with increasing arm lengths will be synthesized either chemically in one or two pads (see above and see Mamone, U.S. Ser. No. 07/882,689, filed May 11, 1992, hereby incorporated by reference herein) or by in vitro transcription (see Cech et al., U.S. Pat. No. 4,987,071). Ribozymes are chemically synthesized with modifications that prevent their degradation by serum ribonucleases (as described in Example 13, above). When synthesized in two parts, the fragments are ligated or otherwise juxtaposed as described (see original application and Mamone, supra). The effects of the ribozymes on smooth muscle cell proliferation are assessed as in Examples 6 and 12, above. As the length of stems I and III can affect both hybridization to the target and the catalytic rate, the arm length of each ribozyme will be optimized for maximal inhibitory effect in cells. Similarly, the precise sequence of modified nucleotides in the stabilized ribozyme will affect the activity in cells. The nature of the stabilizing modifications will be optimized for maximal inhibitory effect in cells. In each case, activity of the ribozyme that cleaves c-myb RNA will be compared to the activity of its catalytically inactive control (substitution of 2'-O- methyl U for G.sub.5 and a 2'-O-methyl U for A.sub.14) and to a ribozyme targeted to an irrelevant RNA (same catalytic core, with appropriate modifications, but different binding arm sequences).

Detailed Description Text (63):
Sullivan, et al., supra, describes the general methods for delivery of enzymatic RNA molecules. The data presented in Example 9 indicate that different cationic lipids can deliver active ribozymes to rat smooth muscle cells. In this example, 0.6 .mu.M ribozyme delivered with Lipofectamine produced the same inhibitory effect as 0.3 .mu.M ribozyme delivered with DMRIE. Thus, DMRIE is twice as efficacious as Lipofectamine at delivering active ribozymes to smooth muscle cells. There are a number of other cationic lipids known to those skilled in the art that can be used to deliver nucleic acid to cells, including but not limited to dioctadecylamidoglycylspermine (DOGS), dioleoxltrimetylammonium propane (DOTAP), N-[1-(2,3-dioleoyloxy)-propyl]-n,n,n-trimethylammoniumchloride (DOTMA), N-[1-(2,3-dioleoyloxy)-propyl]-N,N-dimethyl-N-hydroxyethylammonium bromide (DORIE), and N-[1-(2,3-dioleoyloxy)propyl]-N,N-dimethyl-N-hydroxypropylammonium bromide (DORIE-HP). Experiments similar to those performed in Example 9 are used to determine which lipids give optimal delivery of ribozymes to smooth muscle cells. Other such delivery methods are known in the art and can be utilized in this invention.

Detailed Description Text (65):
The data presented in Example 13 indicate that the proliferation of smooth muscle cells can be inhibited by the direct addition of chemically stabilized ribozymes. Presumably, uptake is mediated by passive diffusion of the anionic nucleic acid across the cell membrane. In this case, efficacy could be greatly enhanced by directly coupling a ligand to the ribozyme. The ribozymes are then delivered to the cells by receptor-mediated uptake. Using such conjugated adducts, cellular uptake can be increased by several orders of magnitude without having to alter the phosphodiester linkages necessary for ribozyme cleavage activity.

Detailed Description Text (69):
As the applicant had shown in Example 13, the hammerhead (HH) ribozyme that cleaves c-myb RNA at site 575 can be modified to confer resistance to nucleases while maintaining catalytic activity (see also Usman et al., supra). To identify ribozymes with optimal activity in cells, several different chemically-modified ribozymes were directly compared for inhibition of rat smooth muscle cell proliferation. Chemically-modified ribozymes used are diagrammed in FIG. 9A. One ribozyme (designated "2'-O-methyl") contains ribonucleotide residues at all positions except the 5 terminal nucleotides of each target binding arm (Stems I and III). The ribozyme designated "2'-O-methyl P=S" in addition contains five phosphorothioate linkages between the terminal nucleotides in each target binding arm. The ribozyme termed "2'-C-allyl iT" contains thirty 2'-O-methyl nucleotides as specified in Example 13. The ribozyme also contains 2'-C-allyl U (Usman et al., 1994 Nucleic Acids Symp. Ser. 31, 163) at the U4 position and 2'-O-methyl U at the U7 position and a 3'-3'-linked inverted thymidine (Ortigao et al., 1992 Antisense Res. & Development 2, 129; Seliger et al., Canadian Patent Application No. 2,106,819) at the 3' end of the molecule (referred to as 2'-C-allyl iT). The fourth ribozyme contains the same 2'-O-methyl and 2'-C-allyl residues described above with the addition of 5 phosphorothioate linkages between the terminal nucleotides in each target binding arm (referred to as "2'-C-allyl P=S").

Detailed Description Text (76):
Ribozymes are thought to be more specific reagents for the inhibition of gene expression than antisense oligonucleotides due to their catalytic activity and strict sequence requirements around the site of cleavage (Castanotto et al., 1994 Adv. in Pharmacol. 25, 289). To test this hypothesis, ribozyme activity was directly compared to the activity of phosphorothioate DNA oligonucleotides that target the same site in the c-myb mRNA. The ribozyme used was the 2'-C-allyl P=S-modified ribozyme described in Example 14, above. This ribozyme binds to a 15 nucleotide long region of the c-myb mRNA. Thus, a 15 nucleotide antisense phosphorothioate DNA molecule was prepared. A phosphorothioate DNA oligonucleotide with a randomly scrambled sequence of the same 15 nucleotides and a 2'-C-allyl P=S-modified ribozyme with randomly scrambled target binding arm sequences were synthesized as controls (by comparison to the murine c-myb cDNA sequence, the scrambled controls would not be expected to bind any region of the c-myb mRNA). Since longer phosphorothioate DNA oligonucleotides are often utilized as antisense inhibitors (for a review see Wagner, 1994 Science 372, 333), a symmetrically placed, 25 nucleotide phosphorothioate DNA antisense oligonucleotide and its scrambled sequence control were also synthesized. The ribozymes and the antisense oligonucleotides were delivered to rat smooth muscle cells as complexes with the cationic lipid, Lipofectamine, and serum-stimulated smooth muscle cell proliferation was measured subsequently.

Detailed Description Text (80):
If the observed inhibition of smooth muscle cell proliferation is mediated by ribozyme cleavage of c-myb mRNA, then other ribozymes that target the same mRNA should have the same effect. Two other ribozymes targeting two disparate sites in the c-myb mRNA (sites 549 and 1553, ribozyme Seq. ID Nos. 102 and 112) were synthesized with the 2'-C-allyl P=S modifications as described in Example 14. Inactive ribozyme controls also were synthesized corresponding to each new target sequence. Chemically-modified ribozymes targeting sites 549, 575, and 1553 were delivered to rat smooth muscle cells and their ability to inhibit serum-stimulated cell proliferation was assessed. Equivalent levels of inhibition are obtained with active ribozymes targeting sites 549, 575 and 1553 (see FIG. 12). None of the inactive ribozymes inhibited cell proliferation. Active ribozymes targeting other

mRNA sequences not present in c-myb or ribozymes with scrambled arm sequences also fail to inhibit smooth muscle cell proliferation (see FIG. 12). Thus, inhibition of cell proliferation requires a catalytically active ribozyme that can bind to accessible c-myb mRNA sequences and is likely due to the reduction of c-myb mRNA levels by ribozyme cleavage.

Detailed Description Text (91):
Ribozymes that cleave c-myb RNA at position 575 were synthesized with varying arm lengths. Each ribozyme contained 4 phosphorothioate linkages at the 5' end, 2'-O-methyl and 2'-C-allyl modifications and an inverted thymidine nucleotide at the 3' end as described above. FIG. 15 shows the effects of these ribozymes upon rat smooth muscle cell proliferation. Ribozymes were delivered at 100 nM with cationic lipid. Ribozymes with 6/6, 7/7 and 5/10 arms (where x/y denotes the nucleotides in Stem I/nucleotides in Stem III; see FIG. 2) all showed comparable efficacy. As shown in FIG. 15, ribozymes with longer arm lengths tended to demonstrate more non-specific inhibition (the inactive ribozyme controls with longer binding arms inhibited smooth muscle cell proliferation) when compared to ribozymes with shorter binding arms. From these data, it appears that ribozymes with 6/6, 7/7, 5/10, 10/5, 8/8 and 10/10 nucleotide arms all specifically inhibit smooth muscle cell proliferation, optimal inhibition, however, is observed with 6/6, 7/7 and 5/10 nucleotide arms.

Detailed Description Text (101):
In Example 12, we demonstrated that a minimally modified ribozyme directed against c-myb site 549 could significantly inhibit human smooth muscle cell proliferation. The 2'-C-allyl and phosphorothioate-modified ribozyme targeting c-myb site 575 characterized above was applied to human smooth muscle cells as RNA/Lipofectamine.RTM. complexes. Inactive ribozyme and inactive, scrambled arm ribozymes were applied as controls. At 200 nM, the active ribozyme inhibits human smooth muscle proliferation by greater than 75% while the inactive ribozyme inhibits proliferation by only 38%. The ribozyme with scrambled binding arm sequences fails to inhibit. At 100 nM, the active ribozyme still demonstrates significant inhibition while neither the inactive or scramble controls inhibit cell proliferation (see FIG. 18). Thus, the active ribozyme identified in these studies mediates significant inhibition of human smooth muscle cell proliferation and represents a novel therapeutic for restenosis and/or vascular disease.

Detailed Description Text (118):
By "2-5A antisense chimera" is meant, an antisense oligonucleotide containing a 5' phosphorylated 2'-5'-linked adenylate residues. These chimeras bind to target RNA in a sequence-specific manner and activate a cellular 2-5A-dependent ribonuclease which in turn cleaves the target RNA (Torrence et al., 1993 Proc. Natl. Acad. Sci. USA 90, 1300).

Detailed Description Text (128):
Ribozymes of this invention may be used as diagnostic tools to examine genetic drift and mutations within diseased cells or to detect the presence of c-myb RNA in a cell. The close relationship between ribozyme activity and the structure of the target RNA allows the detection of mutations in any region of the molecule which alters the base-pairing and three-dimensional structure of the target RNA. By using multiple ribozymes described in this invention, one may map nucleotide changes which are important to RNA structure and function in vitro, as well as in cells and tissues. Cleavage of target RNAs with ribozymes may be used to inhibit gene expression and define the role (essentially) of specified gene products in the progression of disease. In this manner, other genetic targets may be defined as important mediators of the disease. These experiments will lead to better treatment of the disease progression by affording the possibility of combinational therapies (e.g., multiple ribozymes targeted to different genes, ribozymes coupled with known small molecule inhibitors, or intermittent treatment with combinations of ribozymes and/or other chemical or biological molecules). Other in vitro uses of ribozymes of this invention are well known in the art, and include detection of the presence of mRNAs associated with c-myb related condition. Such RNA is detected by determining the presence of a cleavage product after treatment with a ribozyme using standard methodology.

Detailed Description Text (129):
In a specific example, ribozymes which can cleave only wild-type or mutant forms of the target RNA are used for the assay. The first ribozyme is used to identify wild-type RNA present in the sample and the second ribozyme will be used to identify mutant RNA in the sample. As reaction controls, synthetic substrates of both wild-type and mutant RNA will be cleaved by both ribozymes to demonstrate the relative ribozyme efficiencies in the reactions and the absence of cleavage of the "non-targeted" RNA species. The cleavage products from the synthetic substrates will also serve to generate size markers for the analysis of wild-type and mutant RNAs in the sample population. Thus each analysis will require two ribozymes, two substrates and one unknown sample which will be combined into six reactions. The presence of cleavage products will be determined using an RNAse protection assay so that full-length and cleavage fragments of each RNA can be analyzed in one lane of a polyacrylamide gel. It is not absolutely required to quantify the results to gain insight into the expression of mutant RNAs and putative risk of the desired phenotypic changes in target cells. The expression of mRNA whose protein product is implicated in the development of the phenotype (i.e., c-myb) is adequate to establish risk. If probes of comparable specific activity are used for both transcripts, then a qualitative comparison of RNA levels will be adequate and will decrease the cost of the initial diagnosis. Higher mutant form to wild-type ratios will be correlated with higher risk whether RNA levels are compared qualitatively or quantitatively.

Detailed Description Paragraph Table (1):
TABLE I Characteristics of Ribozymes

Group I Introns Size: .about.200 to >1000 nucleotides. Requires a U in the target sequence immediately 5' of the cleavage site. Binds 4-6 nucletides at 5' side of cleavage site. Over 75 known members of this class. Found in Tetrahymena thermophila rRNA, fungal mitochondria, chloroplasts, phage T4, blue-green algae, and others. RNAseP RNA (M1 RNA) Size: .about.290 to 400 nucleotides. RNA portion of a ribonucleoprotein enzyme. Cleaves tRNA precursors to form mature tRNA. Roughly 10 known members of this group all are bacterial in origin. Hammerhead Ribozyme Size: .about.13 to 40 nucleotides. Requires the target sequence UH immediately 5' of the cleavage site. Binds a variable number nucleotides on both sides of the cleavage site. 14 known members of this class. Found in a number of plant pathogens (virusoids) that use RNA as the infectious agent (FIG. 1) Hairpin Ribozyme Size: .about.50 nucleotides. Requires the target sequence GUC immediately 3' of the cleavage site. Binds 4-6 nucleotides at 5' side of the cleavage site and a variable number to the 3' side of the cleavage site. Only 3 known member of this class. Found in three plant pathogen (satellite RNAs of the tobacco ringspot virus, arabis mosiac virus and chicory yellow mottle virus) which uses RNA as the infectious agent (FIG. 3). Hepatitis Delta Virus (HDV) Ribozyme Size: 50-60 nucleotides (at present). Cleavage of target RNAs recently demonstrated. Sequence requirements not fully determined. Binding sites and structural requirements not fully determined, although no sequences 5' of cleavage site are required. Only 1 known member of this class. Found in human HDV (FIG. 4). Neurospora VS RNA Ribozyme Size: .about.144 nucleotides (at present) Cleavage of target RNAs recently demonstrated. Sequence requirements not fully determined. Binding sites and structural requirements not fully determined. Only 1 known member of this class. Found in Neurospora VS RNA (FIG. 5).

Detailed Description Paragraph Table (2):
TABLE II

Human c-myb Target Sequence Site	Target Sequence	Sequence I.D. No.
86		
GGCGGCAGCGCCCUGCCGACGCCGGGG	ID. NO. 01	162
CCGCGGCUCUCGGC	ID. NO. 02	195
GCCAUGGCCCGAA	ID. NO. 03	213
CGGCACAGCAUAUAUAGCAGUGACGAGGA	ID. NO. 04	249
GACUUUGAGAUGUGUGACCAUGACUAUGAUGGG	ID. NO. 05	295
CUGGAAAGCGUC	ID. NO. 06	332
GGAAGAGGAUGAAAAACUGAAGAAG	ID. NO. 07	350
GAAGAACUGGUGGAACAGAAUGGAAC	ID. NO. 08	383
CUGGAAAGUUAUUGCCAA	ID. NO. 09	407
CCCGAAUCGAACAGAUGUGCAG	ID. NO. 10	446
GAAAGUACUAAACCCUGAG	ID. NO. 11	478
CUUGGACCAAAGAAGAAGAUCAGAGAGUGAUA	ID. NO. 12	518
ACAGAAAUACGGUCCGAAACGUUGGUCUG	ID. NO. 13	547
UUAUUGCCAAGCACUUAAAGGGGAGAAUUGGAA	ID. NO. 14	611
GAAUCCAGAAGUUAAGAA	ID. NO. 15	647
GGAAGACAGAAUUAUUUACCAGGCACA	ID. NO. 16	674
CAAGAGACUGGGGAACAGAU	ID. NO. 17	700
AAAUCGCAAAGCUA	ID. NO. 18	720
GGACGAACUGAUAAUGCUAUCAAGAACC	ID. NO. 19	748
ACUGGAAUUCUACAAUGCGUCGGAAGGUCGAACA	ID.	

NO. 20 816 CAGCCAGCAGUGGCCACAA ID. NO. 21 852 CAUUUGAUGGGUUUUGCUCAGGCUCCGCCUACA ID. NO. 22 885 GCUCAACUCCCUGCCACUGGCCAGCCC ID. NO. 23 918 AACAACGACUAUUCCUAUUACCACA ID. NO. 24 954 CAAAAUGUCUCCAGUCAUGUUCCAUACCCU ID. NO. 25 998 AAAUAUAGUCAAUGUCCCUCAGCCAGCUGCCGCA ID. NO. 26 1039 AGAGACACUAUAAUGAUGAAGACCCUGAGAAGGA ID. NO. 27 1073 AAAGCGAAUAAAGGAAUUAGAAUUG ID. NO. 28 1098 CUCCUAAUGUCAACCGA ID. NO. 29 1120 AGCUAAAAGGACAGCAGGUGCUACCAACACAGAA ID. NO. 30 1161 CCCGGGUGGCACAGCACCACCAUUGCCGACCACA ID. NO. 31 1237 AACACCACUCCACUCCAUCUCUGCCAGCGGAUCC ID. NO. 32 1279 UACCUGAAGAAA ID. NO. 33 1311 AUGAUCGUCCACCAGGGCACCAUU ID. NO. 34 1366 CAGAAACACUCCAAUUUA ID. NO. 35 1418 AAACUCAGACU ID. NO. 36 1434 AUGCCUUCUUUAAC ID. NO. 37 1480 UUACAACACCA ID. NO. 38 1515 ACUCAAAAGGAAAAUACUGUUUUUAGAACCC ID. NO. 39 1546 CAGCUAUCAAAAGGUCAAUCUUAGAAAGCU ID. NO. 40 1576 CUCCAAGAACUCCUACACCAUUCAA ID. NO. 41 1601 ACAUGCACUUGCAGCUCAAGAA ID. NO. 42 1630 UACGGUCCCCUGAAGAUGCUACCUCAGA ID. NO. 43 1657 CACCCUCUCAUCUAGUAGAAGAUCUGCAGGA ID. NO. 44 1693 UCAAACAGGAAUCUGAUGAAUCUGGA ID. NO. 45 1735 AAGAAAAUGGA ID. NO. 46 1751 CUUACUGAAGAAAAUCAAACAAGA ID. NO. 47 1780 AAUCUCCAACUGAUAAAUCAG ID. NO. 48 1813 GCUCACACCACUGGGA ID. NO. 49 1864 CCUCGCCUGUGCGAGAUGCACCGAAUAUUC ID. NO. 50 1913 GGCACCAGCAUCAGAAGAUGAAGAC ID. NO. 51 1951 CAUUUACAGUACC ID. NO. 52 1975 CCCUGGCGAGCCCCUUGCA ID. NO. 53 1994 GCCUUGUAGCAGUACCUGGGA ID. NO. 54 2059 GUCAAGCUCGUAAAUACGUGAA ID. NO. 55 2142 GAACAGUUCAA ID. NO. 56 2181 AUGAAACUUUUCAU ID. NO. 57 2304 AAAAUAAAUAACAGUC ID. NO. 58 2340 UGAAUUGUAGCC ID. NO. 59 2357 UUAAUAUCUUAAU ID. NO. 60 2399 AUUUAUCUGGUAUUUUAAAGGAUCCAACAGAUC ID. NO. 6,1 2483 CCAGUAUUUCA ID. NO. 62 2499 CUCGAUCACUAAACAUAUG ID. NO. 63 2518 CAUAUAUUUUUAAAAAUC ID. NO. 64 2767 UGCUAUGGUCUUAGCCU ID. NO. 65 2799 AGUAUCAGAGG ID. NO. 66 2849 UAGGUAAUUGACUAU ID. NO. 67 2871 UAUUUCAGACUUUUAAUUUUAUAUAUAUAUACA ID. NO. 68 2920 CAAUACAUUUGAAAACUUGUUUGGGAGACUCUGC ID. NO. 69 2964 GUGGUUUUUUUGUUAUUGUUGGUUU ID. NO. 70 3008 UUCUUUUUUGGGAGAU ID. NO. 71 3040 CUAUGUUUUGUUUUG ID. NO. 72 3060 AGCCUGACUGUUUUAUA ID. NO. 73 3089 UCGAUUUGAUC ID. NO. 74 3145 UGGAUCCUGUGUU ID. NO. 75 3184 UUGAUAGCCAGUCACUGCCUUAAGA ID. NO. 76 3209 ACAUUUGAUGCAAGAUGGCCAGCACU ID. NO. 77 3252 CGGUGUACUUACUGCC ID. NO. 78

Detailed Description Paragraph Table (3):

TABLE III

Sequences of ribozymes used in these studies. Target Sequence Site ID No. Ribozyme Sequence

Hammerhead ribozymes with 7 nucleotide binding arms 310 101 UUUCCCCCUGAUGAGGCCGAAAGGCCGAAAGUGACG 549 102 UUGGCAACUGAUGAGGCCGAAAGGCCGAAAACAGAC 551 103 GCUUGGCCUGAUGAGGCCGAAAGGCCGAAAUAACAG 575 104 GCUUUCCCUGAUGAGGCCGAAAGGCCGAAAUUCUCC 634 105 UGUCCAGCUGAUGAGGCCGAAAGGCCGAAAGGUUUU 738 106 UUCUUGACUGAUGAGGCCGAAAGGCCGAAAGCAUUA 839 107 UCUUCUGCUGAUGAGGCCGAAAGGCCGAAAAGCUCG 936 108 AUGUGGUCUGAUGAGGCCGAAAGGCCGAAAUAGGAA 1017 109 GCCGGCUCUGAUGAGCGCGAAAGCGCGAAAGGGACG 1082 110 GCUCCUUCUGAUGAGGCCGAAAGGCCGAAAUUCGCU 1363 111 UUCUGCACUGAUGAGGCCGAAAGGCCGAAAUUCUAA 1553 112 ACCUUUUCUGAUGAGGCCGAAAGGCCGAAAUAGCUG 1597 113 AUGUUUGCUGAUGAGGCCGAAAGGCCGAAAUGGUGU 1598 114 CAUGUUUCUGAUGAGGCCGAAAGGCCGAAAAUGGUG 1635 115 UUCAGGGCUGAUGAGGCCGAAAGGCCGAAACCGUAU 1721 116 CAGCAACCUGAUGAGGCCGAAAGGCCGAAAUUCCAG 1724 117 ACUCAGCCUGAUGAGGCCGAAAGGCCGAAACAAUUC 1895 118 AGCUUGUCUGAUGAGGCCGAAAGGCCGAAAGAAUAU 1909 119 UGUCAUUCUGAUGAGGCCGAAAGGCCGAAAAACAGA 1943 120 CUUUGAGCUGAUGAGGCCGAAAGGCCGAAACAUUGU

Bimolecular Hairpin Ribozymes 1632.sup.a 121 5' Fragment: UCAGGGAGAAGUAUACCAGAGAAACACACGCG 3' Fragment: CGCGUGGUACAUUACCUGGUA 2231.sup.a 122 5' Fragment: GCUCUCAGAAGUUGACCAGAGAAACACACGCG 3' Fragment: CGCGUGGUACAUUACCUGGUA

Hammerhead riboyzmes with 6, 8, 9, 10, and 12 nucleotide binding arms 575 123 CUUUCCCUGAUGAGGCCGAAAGGCCGAA AUUCUC 6/6.sup.b 575 124 UGUUUCCCUGAUGAGGCCGAAAGGCCGAA AUUCUCCC 8/8 575 125 CUGCUUUCCCUGAUGAGGCCGAAAGGCCGAA AUUCUCCCU 9/9 575 126 ACUGCUUUCCCUGAUGAGGCCGAAAGGCCGAA AUUCUCCCUU 10/10 575 127 ACACUGCUUUCCCUGAUGAGGCCGAAAGGCCGAA AUUCUCCCUUUU 12/12 549 128 AGUGCUUGGCAACUGAUGAGGCCGAAAGGCCGAA AACAGACCAACG 12/12 1553 129 GAUUGACCUUUUCUGAUGAGGCCGAAAGGCCGAA AUAGCUGGAGUU 12/12

.sup.a

The hairpin ribozymes were synthesized in two pieces as indicated. The two oligonucleotides were annealed and tested for activity against th cmyb RNA as described above. See Mamone, Ribozyme synthesis, filed May 11 1992, U.S.S.N. 07/882,689, hereby incorporated by reference herein. .sup.b Designation of the

ribozymes with different arm lengths is a/b where (a) represents the nucleotides in stem I and (b) represents the nucleotides in stem III (see FIG. 1).

Detailed Description Paragraph Table (4):

TABLE IV Ribozyme catalyzed cleavage of c-myb RNA % Cleavage Mouse Human Cleavage Sequence c-myb c-myb Site ID No. Target sequence RNA RNA Hammerhead Sites 310 79 CGUCACU U GGGGAAA 28.5 0.1 549 80 GUCUGUU A UUGCCAA 87.4 91.6 551 81 CUGUUAU U GCCAAGC 56.8 82.4 575 82 GGAGAAU U GGAAAAC 93.9 91.3 634 83 AAAACCU C CUGGACA 68.4 87.1 738 84 UAAUGCU A UCAAGAA 78.1 0.01 839 85 CAAGCUU C CAGAAGA 27.2 0.01 936 86 UUCCUAU U ACCACAU 61.8 60.6 1017 97 UGUCCCU C AGCCAGC 40.3 0.1 1082 88 AGCGAAU A AAGGAAU 55.2 89.2 1363 89 UUAGAAU U UGCAGAA 11.6 0.1 1553 90 CAGCUAU C AAAAGGU 87.1 92.5 1597 91 ACACCAU U CAAACAU 71.2 62.7 1598 92 CACCAUU C AAACAUG 79.6 85.5 1635 93 AUACGGU C CCCUGAA 84.4 82.3 1721 94 CUGGAAU U GUUGCUG 62.1 79.3 1724 95 GAAUUGU U GCUGAGU 65.6 86 1895 96 AUAUUCU U ACAAGCU 79.1 66.2 1909 97 UCCGUUU U AAUGGCA 31.1 0.1 1943 98 ACAAUGU U CUCAAAG 66.1 80 Hairpin Ribozymes 1632 99 ACG GUCC CCUGAAG 92.8 84.6 2231 100 ACA GUUG AGAGCAG 0.1 0.1 .sup.a The nucleotide numbers given correspond to the nucleotide just 5' of the ribozyme cleavage site in the human cmyb sequence taken from Westin, et al., supra (GenBank Accession No. X52125). All but two of the sequences (310; I.D. No. 79 and 2231; I.D. No. 100) overlap sequences in Table I.

Detailed Description Paragraph Table (5):

TABLE V Comparison of the effects six hammerhead ribozymes, that cleave c-myb RNA, on smooth muscle cell proliferation. Inactive Ribozyme Active Ribozyme Ribozyme % Cell % Cell % Inhibition Site Proliferation Proliferation (Active vs. Inactive) 549 68 .+-. 1 59.5 .+-. 1.5 14 .+-. 4 575 66.5 .+-. 0.5 54.5 .+-. 1.5 21 .+-. 3 1553 68.5 .+-. 0.5 52 .+-. 1 28 .+-. 1 1597 66 .+-. 1 57 .+-. 3 16 .+-. 7 1598 67 .+-. 1 58.5 .+-. 0.5 1.5 .+-. 1 1635 62.5 .+-. 2.5 64 .+-. 1 0

Detailed Description Paragraph Table (7):

TABLE VII Dose Response of c-myb Hammerhead Ribozymes 575 and 549 Control Ribozyme 575 Ribozyme 549 Ribozyme % Inhibi- % Inhibi- Ribozyme % cells % cells tion (vs. % cells tion (vs. Dose (.mu.M) in S phase in S phase control) in S phase control) 0.05 89 .+-. 5 77.5 .+-. 1.5 14 .+-. 8 92 .+-. 1 0 0.15 90 .+-. 1 68.5 .+-. 1.5 26 .+-. 2 84 .+-. 2 9 .+-. 4 0.45 91.5 .+-. 0.5 59 .+-. 5 38 .+-. 7 76.5 .+-. 2.5 18 .+-. 5

Detailed Description Paragraph Table (9):

TABLE IX Arm Length Variations of c-myb Hammerhead Ribozyme 575 Arm Length % Inhibition (base-pairs) % cells in S phase (vs. Inactive 7/7) 6/6 62 .+-. 1 4 .+-. 4 7/7 60 .+-. 1 7 .+-. 3 8/8 60.5 .+-. 0.5 6 .+-. 2 9/9 53.5 .+-. 0.5 18 .+-. 2 10/10 55 .+-. 1 16 .+-. 4 12/12 48 .+-. 1 28 .+-. 3

Detailed Description Paragraph Table (10):

TABLE X Hammerhead ribozymes with 7 vs. 12-nucleotide binding arms targeting three different sites. Inactive Active Ribozyme Length of Ribozyme Ribozyme % Inhibition Target Binding (% Cell (% Cell (Active vs. Site Arms Proliferation) Proliferation) Inactive) 575 7/7 51.5 .+-. 0.5 43 .+-. 0.5 24 .+-. 5 575 12/12 50.5 .+-. 3.5 37 .+-. 0.5 37 .+-. 4 549 7/7 49.5 .+-. 0.5 44.5 .+-. 1.5 21 .+-. 7 659 12/12 48.5 .+-. 1.5 35 .+-. 2 41 .+-. 7 1553 7/7 49.5 .+-. 0.5 43.5 .+-. 2.5 23 .+-. 9 1553 12/12 49 .+-. 1 33.5 .+-. 1.5 45 .+-. 6

Detailed Description Paragraph Table (14):

TABLE XIV

Human c-myb Hairpin Ribozyme and Target Sequences	Position	Ribozyme Sequenc	Target
CCCUCCCC AGAA GCGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA GCGCA GCC GGGGAGGG	104		148
ACCGACCG AGAA GCCG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA CGGCA GCC CGGUCGGU			185

GCGCGGCG AGAA GCGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA CCGCC GCC CGCCGCGC 528
ACGUUUCG AGAA GUAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AUACG GUC CGAAACGU 715
UUCGUCCA AGAA GUAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA CUACU GCC UGGACGAA 1025
AUGGCUGC AGAA GCUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA CAGCU GCC GCAGCCAU 1187
CUGGUGUG AGAA GCAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA UUGCC GAC CACACCAG 1532
GUUCUAAA AGAA GUAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AUACU GUU UUUAGAAC 1632
CUUCAGGG AGAA GUAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AUACG GUC CCCUGAAG 1836
GGUAUUCA AGAA GUCC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA GGACA GUC UGAAUACC 1852
UCUGCGUG AGAA GUUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA CAACU GUU CACGCAGA 1861
CAGGCGAG AGAA GCGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA ACGCA GAC CUCGCCUG 1993
UGCUACAA AGAA GCAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA UUGCA GCC UUGUAGCA 2231
CUGCUCUC AGAA GUUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA CAACA GUU GAGAGCAG 2316
UUAGGUAA AGAA GUUA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA UAACA GUC UUACCUAA 3068
AAUUAUAA AGAA GUCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA UGACU GUU UUAUAAUU 3138
AUCCAUGC AGAA GUUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA GAACU GUU GCAUGGAU 3199
GUUCUUAA AGAA GUGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA UCACU GCC UUAAGAAC 3264
UGCUACAA AGAA GUAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA UUACU GCC UUGUAGCA

Detailed Description Paragraph Table (15):

TABLE XV

Human c-myb Hammerhead Ribozyme and Target Sequences (REVISED) Seq. Seq. nt. Target Seqence ID No. HH Ribozyme Sequence ID No. 15

AACCUGUU U CCUCCUCC 170 GGAGGAGG CUGAUGAGGCCGAAAGGCCGAA 171AGGUU 16 ACCUGUUU C
CUCCUCCU 172 AGGAGGAG CUGAUGAGGCCGAAAGGCCGAA 173CAGGU 19 UGUUUCCU C CUCCUCCU 174
AGGAGGAG CUGAUGAGGCCGAAAGGCCGAA 175AAACA 22 UUCCUCCU C CUCCUUCU 176 AGAAGGAG
CUGAUGAGGCCGAAAGGCCGAA 177AGGAA 25 CUCCUCCU C CUUCUCCU 178 AGGAGAAG
CUGAUGAGGCCGAAAGGCCGAA 179AGGAG 28 CUCCUCCU U CUCCUCCU 180 AGGAGGAG
CUGAUGAGGCCGAAAGGCCGAA 181AGGAG 29 UCCUCCUU C UCCUCCUC 182 GAGGAGGA
CUGAUGAGGCCGAAAGGCCGAA 183GAGGA 31 CUCCUUCU C CUCCUCCU 184 AGGAGGAG
CUGAUGAGGCCGAAAGGCCGAA 185AGGAG 34 CUUCUCCU C CUCCUCCG 186 CGGAGGAG
CUGAUGAGGCCGAAAGGCCGAA 187AGAAG 37 CUCCUCCU C CUCCGUCA 188 UCACGGAG
CUGAUGAGGCCGAAAGGCCGAA 189AGGAG 40 CUCCUCCU C CGUCACCU 190 AGGUCACG
CUGAUGAGGCCGAAAGGCCGAA 191AGGAG 49 CGUGACCU C CUCCUCCU 192 AGGAGGAG
CUGAUGAGGCCGAAAGGCCGAA 193UCACG 52 GACCUCCU C CUCCUCUU 194 AAGAGGAG
CUGAUGAGGCCGAAAGGCCGAA 195AGGUC 55 CUCCUCCU C CUCUUUCU 196 AGAAAGAG
CUGAUGAGGCCGAAAGGCCGAA 197AGGAG 58 CUCCUCCU C UUUCUCCU 198 AGGAGAAA
CUGAUGAGGCCGAAAGGCCGAA 199AGGAG 60 CCUCCUCU U UCUCCUGA 200 UCAGGAGA
CUGAUGAGGCCGAAAGGCCGAA 201GGAGG 61 CUCCUCUU U CUCCUGAG 202 CUCAGGAG
CUGAUGAGGCCGAAAGGCCGAA 203AGGAG 62 UCCUCUUU C UCCUGAGA 204 UCUCAGGA
CUGAUGAGGCCGAAAGGCCGAA 205GAGGA 64 CUCUUUCU C CUGAGAAA 206 UUUCUCAG
CUGAUGAGGCCGAAAGGCCGAA 207AAGAG 75 GAGAAACU U CGCCCCAG 208 CUGGGGCG
CUGAUGAGGCCGAAAGGCCGAA 209UUCUC 76 AGAAACUU C GCCCCAGC 210 GCUGGGGC
CUGAUGAGGCCGAAAGGCCGAA 211UUUCU 170 CCGCGGCU C UCGCGGAG 212 CUCCGCGA
CUGAUGAGGCCGAAAGGCCGAA 213CGCGG 172 GCGGCUCU C GCGGAGCC 214 GGCUCCGC
CUGAUGAGGCCGAAAGGCCGAA 215GCCGC 224 CACAGCAU A UAUAGCAG 216 CUGCUAUA
CUGAUGAGGCCGAAAGGCCGAA 217CUGUG 226 CAGCAUAU A UAGCAGUG 218 CACUGCUA
CUGAUGAGGCCGAAAGGCCGAA 219UGCUG 228 GCAUAUAU A GCAGUGAC 220 GUCACUGC
CUGAUGAGGCCGAAAGGCCGAA 221UAUGC 253 UGAGGACU U UGAGAUGU 222 ACAUCUCA
CUGAUGAGGCCGAAAGGCCGAA 223CCUCA 254 GAGGACUU U GAGAUGUG 224 CACAUCUC
CUGAUGAGGCCGAAAGGCCGAA 225UCCUC 274 CCAUGACU A UGAUGGGC 226 GCCCAUCA
CUGAUGAGGCCGAAAGGCCGAA 227CAUGG 287 GGGCUGCU U CCCAAGUC 228 GACUUGGG
CUGAUGAGGCCGAAAGGCCGAA 229AGCCC 288 GGCUGCUU C CCAAGUCU 230 AGACUUGG
CUGAUGAGGCCGAAAGGCCGAA 231CAGCC 310 GCGUCAUC U GGGGAAAA 232 UUUUCCCC
CUGAUGAGGCCGAAAGGCCGAA 233GACGC 393 GGAAAGUU A UUGCCAAU 234 AUUGGCAA
CUGAUGAGGCCGAAAGGCCGAA 235UUUCC 395 AAAGUUAU U GCCAAUUA 236 UAAUUGGC
CUGAUGAGGCCGAAAGGCCGAA 237ACUUU 402 UUGCCAAU U AUCUCCCG 238 CGGGAGAU
CUGAUGAGGCCGAAAGGCCGAA 239GGCAA 403 UGCCAAUU A UCCUCCGA 240 UCGGGAGA
CUGAUGAGGCCGAAAGGCCGAA 241UGGCA 405 CCAAUUAU C UCCCGAAU 242 AUUCGGGA
CUGAUGAGGCCGAAAGGCCGAA 243AUUGG 497 AAUUAUCU C CCGAAUCG 244 CGAUUCGG
CUGAUGAGGCCGAAAGGCCGAA 245UAAUU 414 UCCCGAAU C GAACAGAU 246 AUCUGUUC
CUGAUGAGGCCGAAAGGCCGAA 247CGGGA 455 AAAGUACU A AACCCUGA 248 UCAGGGUU
CUGAUGAGGCCGAAAGGCCGAA 249ACUUU 467 CCUGAGCU C AUCAAGGG 250 CCCUUGAU
CUGAUGAGGCCGAAAGGCCGAA 251UCAGG 470 GAGCUCAU C AAGGGUCC 252 GGACCCUU

CUGAUGAGGCCGAAAGGCCGAA 253AGCUC 480 AGGGUCCU U GGACCAAA 254 UUUGGUCC
CUGAUGAGGCCGAAAGGCCGAA 255ACCCU 498 AAGAAGAU C AGAGAGUG 256 CACUCUCU
CUGAUGAGGCCGAAAGGCCGAA 257UUCUU 509 AGAGUGAU A GAGCUUGU 258 ACAAGCUC
CUGAUGAGGCCGAAAGGCCGAA 259ACUCU 515 AUAGAGCU U GUACAGAA 260 UUCUGUAC
CUGAUGAGGCCGAAAGGCCGAA 261UCUAU 526 ACAGAAAU A CGGUCCGA 262 UCGGACCG
CUGAUGAGGCCGAAAGGCCGAA 263UCUGU 549 GGUCUGUU A UUGCCAAG 264 CUUGGCAA
CUGAUGAGGCCGAAAGGCCGAA 265AGACC 551 UCUGUUAU U GCCAAGCA 266 UGCUUGGC
CUGAUGAGGCCGAAAGGCCGAA 267ACAGA 562 CAAGCACU U AAAGGGGA 268 UCCCCUUU
CUGAUGAGGCCGAAAGGCCGAA 269GCUUG 563 AAGCACUU A AAGGGGAG 270 CUCCCCUU
CUGAUGAGGCCGAAAGGCCGAA 271UGCUU 575 GGGAGAAU U GGAAAACA 272 UGUUUUCC
CUGAUGAGGCCGAAAGGCCGAA 273AUCCC 603 GGUGGCAU A ACCACUUG 274 CAAGUGGU
CUGAUGAGGCCGAAAGGCCGAA 275CCACC 610 UAACCACU U GAAUCCAG 276 CUGGAUUC
CUGAUGAGGCCGAAAGGCCGAA 277GGUUA 615 ACUUGAAU C CAGAAGUU 278 AACUUCUG
CUGAUGAGGCCGAAAGGCCGAA 279CAAGU 624 CAGAAGUU A AGAAAACC 280 GGUUUUCU
CUGAUGAGGCCGAAAGGCCGAA 281UUCUG 634 GAAAACCU C CUGGACAG 282 CUGUCCAG
CUGAUGAGGCCGAAAGGCCGAA 283UUUUC 659 GACAGAAU U AUUUACCA 284 UGGUAAAU
CUGAUGAGGCCGAAAGGCCGAA 285CUGUC 660 ACAGAAUU A UUUACCAG 286 CUGGUAAA
CUGAUGAGGCCGAAAGGCCGAA 287UCUGU 662 AGAAUUAU U UACCAGGC 288 GCCUGGUA
CUGAUGAGGCCGAAAGGCCGAA 289AUUCU 663 GAAUUAUU U ACCAGGCA 290 UGCCUGGU
CUGAUGAGGCCGAAAGGCCGAA 291AAUUC 664 AAUUAUUU A CCAGGCAC 292 GUGCCUGG
CUGAUGAGGCCGAAAGGCCGAA 293UAAUU 704 GCAGAAAU C GCAAAGCU 294 AGCUUUGC
CUGAUGAGGCCGAAAGGCCGAA 295UCUGC 713 GCAAAGCU A CUGCCUGG 296 CCAGGCAG
CUGAUGAGGCCGAAAGGCCGAA 297UUUGC 732 GAACUGAU A AUGCUAUC 298 GAUAGCAU
CUGAUGAGGCCGAAAGGCCGAA 299AGUUC 738 AUAAUGCU A UCAAGAAC 300 GUUCUUGA
CUGAUGAGGCCGAAAGGCCGAA 301AUUAU 740 AAUGCUAU C AAGAACCA 302 UGGUUCUU
CUGAUGAGGCCGAAAGGCCGAA 303GCAUU 756 ACUGGAAU U CUACAAUG 304 CAUUGUAG
CUGAUGAGGCCGAAAGGCCGAA 305CCAGU 757 CUGGAAUU C UACAAUGC 306 GCAUUGUA
CUGAUGAGGCCGAAAGGCCGAA 307UCCAG 759 GGAAUUCU A CAAUGCGU 308 ACGCAUUG
CUGAUGAGGCCGAAAGGCCGAA 309AUUCC 790 GGAAGGUU A UCUGCAGG 310 CCUGCAGA
CUGAUGAGGCCGAAAGGCCGAA 311CUUCC 792 AAGGUUAU C UGCAGGAG 312 CUCCUGCA
CUGAUGAGGCCGAAAGGCCGAA 313ACCUU 804 AGGAGUCU U CAAAAGCC 314 GGCUUUUG
CUGAUGAGGCCGAAAGGCCGAA 315CUCCU 805 GGAGUCUU C AAAAGCCA 316 UGGCUUUU
CUGAUGAGGCCGAAAGGCCGAA 317ACUCC 838 CACAAGCU U CCAGAAGA 318 UCUUCUGG
CUGAUGAGGCCGAAAGGCCGAA 319UUGUG 839 ACAAGCUU C CAGAAGAA 320 UUCUUCUG
CUGAUGAGGCCGAAAGGCCGAA 321CUUGU 855 ACAGUCAU U UGAUGGGU 322 ACCCAUCA
CUGAUGAGGCCGAAAGGCCGAA 323ACUGU 856 CAGUCAUU U GAUGGGUU 324 AACCCAUC
CUGAUGAGGCCGAAAGGCCGAA 325GACUG 865 GAUGGGUU U UGCUCAGG 326 CCUGAGCA
CUGAUGAGGCCGAAAGGCCGAA 327CCAUC 866 AUGGGUUU U GCUCAGGC 328 GCCUGAGC
CUGAUGAGGCCGAAAGGCCGAA 329CCCAU 870 GUUUUGCU C AGGCUCCG 330 CGGAGCCU
CUGAUGAGGCCGAAAGGCCGAA

Detailed Description Paragraph Table (23):

TABLE XVI

Mouse c-myb Hammerhead Ribozyme and Target Sequences (REVISED) Seq. Seq. nt. HH
Ribozyme Sequence ID No. Target ID No. 10

UCCGCCAA CUGAUGAGGCCGAAAGGCCGAA AGCCCCGG 1226 CCGGGGCUC UUGGCGGA 1227 12 GCUCCGCC
CUGAUGAGGCCGAAAGGCCGAA AGAGCCCC 1228 GGGGCUCUU GGCGGAGC 1229 33 GCCAUGGC
CUGAUGAGGCCGAAAGGCCGAA AGGCGGGC 1230 GCCCGCCUC GCCAUGGC 1231 63 CUACUGUA
CUGAUGAGGCCGAAAGGCCGAA AUGCUGUG 1232 CACAGCAUC UACAGUAG 1233 65 CGCUACUG
CUGAUGAGGCCGAAAGGCCGAA AGAUGCUG 1234 CAGCAUCUA CAGUAGCG 1235 70 UUCAUCGC
CUGAUGAGGCCGAAAGGCCGAA ACUGUAGA 1236 UCUACAGUA GCGAUGAA 1237 93 CACAUCUC
CUGAUGAGGCCGAAAGGCCGAA AUGUCUUC 1238 GAAGACAUU GAGAUGUG 1239 113 GCCCAUCG
CUGAUGAGGCCGAAAGGCCGAA AGUCAUGG 1240 CCAUGACUA CGAUGGGC 1241 134 GCUUUCCA
CUGAUGAGGCCGAAAGGCCGAA AUUUGGGC 1242 GCCCAAAUC UGGAAAGC 1243 145 CCCCAAGU
CUGAUGAGGCCGAAAGGCCGAA ACGCUUUC 1244 GAAAGCGUC ACUUGGGG 1245 149 UUUUCCCC
CUGAUGAGGCCGAAAGGCCGAA AGUGACGC 1246 GCGUCACUU GGGGAAAA 1247 160 UGUCCACC
CUGAUGAGGCCGAAAGGCCGAA AGUUUUCC 1248 GGAAAACUA GGUGGACA 1249 231 UUGGCAAU
CUGAUGAGGCCGAAAGGCCGAA ACUUUCCA 1250 UGGAAAGUC AUUGCCAA 1251 234 UAAUUGGC
CUGAUGAGGCCGAAAGGCCGAA AUGACUUU 1252 AAAGUCAUU GCCAAUUA 1253 241 GGGCAGAU
CUGAUGAGGCCGAAAGGCCGAA AUUGGCAA 1254 UUGCCAAUU AUCUGCCC 1255 242 UGGGCAGA
CUGAUGAGGCCGAAAGGCCGAA AAUUGGCA 1256 UGCCAAUUA UCUGCCCA 1257 244 GUUGGGCA
CUGAUGAGGCCGAAAGGCCGAA AUAAUUGG 1258 CCAAUUAUC UGCCCAAC 1259 264 UGGCACUG
CUGAUGAGGCCGAAAGGCCGAA ACAUCUGU 1260 ACAGAUGUA CAGUGCCA 1261 306 CCUUUGAU
CUGAUGAGGCCGAAAGGCCGAA AGUUCAGG 1262 CCUGAACAC AUCAAAGG 1263 309 GGACCUUU

CUGAUGAGGCCGAAAGGCCGAA	AUGAGUUC	1264	GAACUCAUC	AAAGGUCC	1265	316	GGUCCAGG
CUGAUGAGGCCGAAAGGCCGAA	ACCUUUGA	1266	UCAAAGGUC	CCUGGACC	1267	337	GACUCUCU
CUGAUGAGGCCGAAAGGCCGAA	AUCUUCUU	1268	AAGAAGAUC	AGAGAGUC	1269	345	AGCUUUAU
CUGAUGAGGCCGAAAGGCCGAA	ACUCUCUG	1270	CAGAGAGUC	AUAAAGCU	1271	348	ACAAGCUU
CUGAUGAGGCCGAAAGGCCGAA	AUGACUCU	1272	AGAGUCAUA	AAGCUUGU	1273	354	UUCUGGAC
CUGAUGAGGCCGAAAGGCCGAA	AGCUUUAU	1274	AUAAAGCUU	GUCCAGAA	1275	357	UAUUUCUG
CUGAUGAGGCCGAAAGGCCGAA	ACAAGCUU	1276	AAGCUUGUC	CAGAAAUA	1277	365	UCGGACCA
CUGAUGAGGCCGAAAGGCCGAA	AUUUCUGG	1278	CCAGAAAUA	UGGUCCGA	1279	370	ACGCUUCG
CUGAUGAGGCCGAAAGGCCGAA	ACCAUAUU	1280	AAUAUGGUC	CGAAGCGU	1281	379	AACAGACC
CUGAUGAGGCCGAAAGGCCGAA	ACGCUUCG	1282	CGAAGCGUU	GGUCUGUU	1283	383	CAAUAACA
CUGAUGAGGCCGAAAGGCCGAA	ACCAACGC	1284	GCGUUGGUC	UGUUAUUG	1285	387	UUGGCAAU
CUGAUGAGGCCGAAAGGCCGAA	ACAGACCA	1286	UGGUCUGUU	AUUGCCAA	1287	388	CUUGGCAA
CUGAUGAGGCCGAAAGGCCGAA	AACAGACC	1288	GGUCUGUUA	UUGCCAAG	1289	390	UGCUUGGC
CUGAUGAGGCCGAAAGGCCGAA	AUAACAGA	1290	UCUGUUAUU	GCCAAGCA	1291	401	UCCCUUUU
CUGAUGAGGCCGAAAGGCCGAA	AGUGCUUG	1292	CAAGCACUU	AAAAGGGA	1293	402	CUCCCUUU
CUGAUGAGGCCGAAAGGCCGAA	AAGUGCUU	1294	AAGCACUUA	AAAGGGAG	1295	414	UGCUUUCC
CUGAUGAGGCCGAAAGGCCGAA	AUUCUCCC	1296	GGGAGAAUU	GGAAAGCA	1297	427	CCUCUCCC
CUGAUGAGGCCGAAAGGCCGAA	ACACUGCU	1298	AGCAGUGUC	GGGAGAGG	1299	448	UGGAUUCA
CUGAUGAGGCCGAAAGGCCGAA	AUGGUUGA	1300	ACAACCAUU	UGAAUCCA	1301	449	CUGGAUUC
CUGAUGAGGCCGAAAGGCCGAA	AAUGGUUG	1302	CAACCAUUU	GAAUCCAG	1303	454	AACUUCUG
CUGAUGAGGCCGAAAGGCCGAA	AUUCAAAU	1304	AUUUGAAUC	CAGAAGUU	1305	462	GUUUUCUU
CUGAUGAGGCCGAAAGGCCGAA	ACUUCUGG	1306	CCAGAAGUU	AAGAAAAC	1307	463	GGUUUUCU
CUGAUGAGGCCGAAAGGCCGAA	AACUUCUG	1308	CAGAAGUUA	AGAAAACC	1309	473	CUGUCCAG
CUGAUGAGGCCGAAAGGCCGAA	AGGUUUUC	1310	GAAAACCUC	CUGGACAG	1311	498	UGGUAAAU
CUGAUGAGGCCGAAAGGCCGAA	AUUCUGUC	1312	GACAGAAUC	AUUUACCA	1313	501	GCCUGGUA
CUGAUGAGGCCGAAAGGCCGAA	AUGAUUCU	1314	AGAAUCAUU	UACCAGGC	1315	502	UGCCUGGU
CUGAUGAGGCCGAAAGGCCGAA	AAUGAUUC	1316	GAAUCAUUU	ACCAGGCA	1317	503	GUGCCUGG
CUGAUGAGGCCGAAAGGCCGAA	AAAUGAUU	1318	AAUCAUUUA	CCAGGCAC	1319	520	GUUCCCCA
CUGAUGAGGCCGAAAGGCCGAA	ACGCUUGU	1320	ACAAGCGUC	UGGGGAAC	1321	543	AGCUUUGC
CUGAUGAGGCCGAAAGGCCGAA	AUCUCUGC	1322	GCAGAGAUC	GCAAAGCU	1323	571	GAUAGCAU
CUGAUGAGGCCGAAAGGCCGAA	AUCAGUCC	1324	GGACUGAUA	AUGCUAUC	1325	577	GUUCUUGA
CUGAUGAGGCCGAAAGGCCGAA	AGCAUUAU	1326	AUAAUGCUA	UCAAGAAC	1327	579	UGGUUCUU
CUGAUGAGGCCGAAAGGCCGAA	AUAGCAUU	1328	AAUGCUAUC	AAGAACCA	1329	595	CAUGGUGG
CUGAUGAGGCCGAAAGGCCGAA	AUUCCAGU	1330	ACUGGAAUU	CCACCAUG	1331	596	GCAUGGUG
CUGAUGAGGCCGAAAGGCCGAA	AAUUCCAG	1332	CUGGAAUUC	CACCAUGC	1333	607	CACCUUGC
CUGAUGAGGCCGAAAGGCCGAA	ACGCAUGG	1334	CCAUGCGUC	GCAAGGUG	1335	629	UCUGCAGG
CUGAUGAGGCCGAAAGGCCGAA	AGCCUUCC	1336	GGAAGGCUA	CCUGCAGA	1337	643	GGCUUUGG
CUGAUGAGGCCGAAAGGCCGAA	AGGCUUCU	1338	AGAAGCCUU	CCAAAGCC	1339	644	UGGCUUUG
CUGAUGAGGCCGAAAGGCCGAA	AAGGCUUC	1340	GAAGCCUUC	CAAAGCCA	1341	677	UCUUCUGG
CUGAUGAGGCCGAAAGGCCGAA	AGCUCGUG	1342	CACGAGCUU	CCAGAAGA	1343	678	UUCUUCUG
CUGAUGAGGCCGAAAGGCCGAA	AAGCUCGU	1344	ACGAGCUUC	CAGAAGAA	1345	691	CAUCAAAU
CUGAUGAGGCCGAAAGGCCGAA	AUUGUUCU	1346	AGAACAAUC	AUUUGAUG	1347	694	CCCCAUCA
CUGAUGAGGCCGAAAGGCCGAA	AUGAUUGU	1348	ACAAUCAUU	UGAUGGGG	1349	695	ACCCCAUC
CUGAUGAGGCCGAAAGGCCGAA	AAUGAUUG	1350	CAAUCAUUU	GAUGGGGU	1351	704	CAUGCCCA
CUGAUGAGGCCGAAAGGCCGAA	ACCCCAUC	1352	GAUGGGGUU	UGGGCAUG	1353	705	GCAUGCCC
CUGAUGAGGCCGAAAGGCCGAA	AACCCCAU	1354	AUGGGGUUU	GGGCAUGC	1355	716	AUGGAGGU
CUGAUGAGGCCGAAAGGCCGAA	AGGCAUGC	1356	GCAUGCCUC	ACCUCCAU	1357	721	CUGAGAUG
CUGAUGAGGCCGAAAGGCCGAA	AGGUGAGG	1358	CCUCACCUC	CAUCUCAG	1359	725	AGAGCUGA
CUGAUGAGGCCGAAAGGCCGAA	AUGGAGGU	1360	ACCUCCAUC	UCAGCUCU	1361	727	AGAGAGCU
CUGAUGAGGCCGAAAGGCCGAA	AGAUGGAG	1362	CUCCAUCUC	AGCUCUCU	1363	732	CUUGGAGA
CUGAUGAGGCCGAAAGGCCGAA	AGCUGAGA	1364	UCUCAGCUC	UCUCCAAG	1365	734	CACUUGGA
CUGAUGAGGCCGAAAGGCCGAA	AGAGCUGA	1366	UCAGCUCUC	UCCAAGUG	1367	736	GCCACUUG
CUGAUGAGGCCGAAAGGCCGAA	AGAGAGCU	1368	AGCUCUCUC	CAAGUGGC	1369	749	UGACGGAG
CUGAUGAGGCCGAAAGGCCGAA	ACUGGCCA	1370	UGGCCAGUC	CUCCGUCA	1371	752	UGUUGACG
CUGAUGAGGCCGAAAGGCCGAA	AGGACUGG	1372	CCAGUCCUC	CGUCAACA	1373	756	UCGCUGUU
CUGAUGAGGCCGAAAGGCCGAA	ACGGAGGA	1374	UCCUCCGUC	AACAGCGA	1375	767	AAUAGGGA
CUGAUGAGGCCGAAAGGCCGAA	AUUCGCUG	1376	CAGCGAAUA	UCCCUAUU	1377	769	GUAAUAGG
CUGAUGAGGCCGAAAGGCCGAA	AUAUUCGC	1378	GCGAAUAUC	CCUAUUAC	1379	773	UGUGGUAA
CUGAUGAGGCCGAAAGGCCGAA	AGGGAUAU	1380	AUAUCCCUA	UUACCACA	1381	775	GAUGUGGU
CUGAUGAGGCCGAAAGGCCGAA	AUAGGGAU	1382	AUCCCUAUU	ACCACAUC	1383	776	CGAUGUGG
CUGAUGAGGCCGAAAGGCCGAA	AAUAGGGA	1384	UCCCUAUUA	CCACAUCG	1385	783	GCUUCGGC
CUGAUGAGGCCGAAAGGCCGAA	AUGUGGUA	1386	UACCACAUC	GCCGAAGC			

Detailed Description Paragraph Table (30):
TABLE XVII - Mouse c-myb Hairpin ribozyme and target sequences (REVISED) Position

Ribozyme Seq. ID No. Substrate Seq. ID No. 24 GCGAGGCG AGAA GGGGCU
ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2182 AGCCCCG GCC CGCCUCGC 2183 28 CAUGGCGA AGAA
GGCCGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2184 CCGGCCC GCC UCGCCAUG 2185 122
AUUUGGGC AGAA GCCCAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2186 AUGGGCU GCU GCCCAAAU
2187 125 CAGAUUUG AGAA GCAGCC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2188 GGCUGCU GCC
CAAAUCUG 2189 216 UUCCAGUC AGAA GUUCCG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2190
CGGAACA GAC GACUGGAA 2191 245 UCCGGUUG AGAA GAUAAU
ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2192 AUUAUCU GCC CAACCGGA 2193 258 CACUGUAC
AGAA GUCCGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2194 CCGGACA GAU GUACAGUG 2195 529
CUCUGCCC AGAA GUUCCC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2196 GGGAACA GAU GGGCAGAG
2197 551 GUCCGGGC AGAA GCUUUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2198 CAAAGCU GCU
GCCCGGAC 2199 554 UCCGUCCG AGAA GCAGCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2200
AGCUGCU GCC CGGACGGA 2201 559 AUCAGUCC AGAA GGGCAG
ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2202 CUGCCCG GAC GGACUGAU 2203 563 CAUUAUCA
AGAA GUCCGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2204 CCGGACG GAC UGAUAAUG 2205 656
CCACUGGC AGAA GGCUGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2206 CCAGCCA GAC GCCAGUGG
2207 728 UUGGAGAG AGAA GAGAUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2208 CAUCUCA GCU
CUCUCCAA 2209 746 UGACGGAG AGAA GGCCAC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2210
GUGGCCA GUC CUCCGUCA 2211 822 UGCAAUGC AGAA GGAUAG
ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2212 CUAUCCU GUC GCAUUGCA 2213 857 CCGCAGCC
AGAA GAGGGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2214 UCCCUCA GCC GGCUGCGG 2215 861
GCUGCCGC AGAA GGCUGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2216 UCAGCCG GCU GCGGCAGC
2217 941 CUGUUGAC AGAA GGAGCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2218 UGCUCCU GAU
GUCAACAG 2218 1040 GAGGUCUG AGAA GGUCCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2220
UGGACCA GAC CAGACCUC 2221 1045 CCCAUGAG AGAA GGUCUG
ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2222 CAGACCA GAC CUCAUGGG 2223 1068 AAACAGGA
AGAA GGUGCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2224 UGCACCU GUU UCCUGUUU 2225 1075
UUCUCCCA AGAA GGAAAC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2226 GUUUCCU GUU UGGGAGAA
2227 1106 GAUCUGCA AGAA GAGAUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2228 CAUCUCU GCC
UGCAGAUC 2229 1113 GAGCCGGG AGAA GCAGGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2230
GCCUGCA GAU CCCGGCUC 2231 1120 AGGUAGGG AGAA GGGAUC
ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2232 GAUCCCG GCU CCCUACCU 2233 1226 AAUCUAUA
AGAA GGAGUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2234 CACUCCA GUU UAUAGAUU 2235 1340
UUUUCACA AGAA GGUCUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2236 GAGACCA GAC UGUGAAAA
2237 1449 AUUUCUUG AGAA GCAAGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2238 CCUUGCA GCU
CAAGAAAU 2239 1468 CUUCAGGG AGAA GUAUUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2240
AAAUACG GUC CCCUGAAG 2241 1490 GGGAGGGG AGAA GAGGUA
ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2242 UACCUCA GAC CCCCUCCC 2243 1542 CCAGAUUC
AGAA GAUUCC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2244 GGAAUCG GAU GAAUCUGG 2245 1648
GUGGUUUG AGAA GAAGAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2246 UUCUUCU GCU CAAACCAC
2247 1672 GGUGCUCA AGAA GUUCUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2248 GAGAACA GCC
UGAGCACC 2249 1688 CCUGCGAG AGAA GUUGGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2250
CCCAACU GUU CUCGCAGG 2251 1713 UUUGGGGC AGAA GCCACA
ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2252 UGUGGCA GAU GCCCCAAA 2253 1740 GUCAUUAA
AGAA GAGCUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2254 AAGCUCU GUU UUAAUGAC 2255 1880
AGGCCGUC AGAA GGUCCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2256 AGGACCA GAU GACGGCCU
2257 1887 GGACCGGA AGAA GUCAUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2258 GAUGACG GCC
UCCGGUCC 2259 1894 CCGAGCCG AGAA GGAGGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2260
GCCUCCG GUC CGGCUCGG 2261 1899 UAUUUCCG AGAA GGACCG
ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2262 CGGUCCG GCU CGGAAAUA 2263 1926 AGAGUUCG
AGAA GAGAAC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2264 GUUCUCA GCU CGAACUCU 2265 2048
ACAACAAA AGAA GGCUCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2266 AGAGCCU GAU UUUGUUGU
2267 2068 CUGCUCUC AGAA GUUGUA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2268 UACAACA GUU
GAGAGCAG 2269 2170 UUAGGUAA AGAA GUUAUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2270
AAUAACA GUC UUACCUAA 2271 2225 UUUAAAAA AGAA GAUUAU
ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2272 AUAAUCA GAU UUUUUAAA 2273 2276 AAAUACUG
AGAA GUUGUA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2274 UACAACA GAU CAGUAUUU 2275 2519
UUCAAGCA AGAA GACAAC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2276 GUUGUCA GCU UGCUUGAA
2277 2717 AGUGCAUA AGAA GUUAUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2278 GAUAACG GAC
UAUGCACU 2279 2737 AUAAAAAA AGAA GGAAUA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2280
UAUUCCA GAC UUUUUUAU 2281

Detailed Description Paragraph Table (31):
TABLE XVIII

Porcine

c-myb (region A) Hammerhead Ribozyme and Target Sequence (266 bp; nt. 458 start; Human numbering system) (REVISED) Position HH Ribozyme Seq. ID No. Substrate Seq. ID No. 467

CCCUUGAU	CUGAUGAGGCCGAAAGGCCGAA	AGAUNAGG	2282	CCUNAUCUC	AUCAAGGG	2283	470 GGACCCUU
CUGAUGAGGCCGAAAGGCCGAA	AUGAGAUN	2284	NAUCUCAUC	AAGGGUCC	2285	477	GGUCCAAG
CUGAUGAGGCCGAAAGGCCGAA	ACCCUUGA	2286	UCAAGGGUC	CUUGGACC	2287	480	UUUGGUCC
CUGAUGAGGCCGAAAGGCCGAA	AGGACCCU	2288	AGGGUCCUU	GGACCAAA	2289	498	CACUCUCU
CUGAUGAGGCCGAAAGGCCGAA	AUCUUCUU	2290	AAGAAGAUC	AGAGAGUG	2291	509	ACAAGCUC
CUGAUGAGGCCGAAAGGCCGAA	AUCACUCU	2292	AGAGUGAUA	GAGCUUGU	2293	515	UUCUGUAC
CUGAUGAGGCCGAAAGGCCGAA	AGCUCUAU	2294	AUAGAGCUU	GUACAGAA	2295	518	UAUUUCUG
CUGAUGAGGCCGAAAGGCCGAA	ACAAGCUC	2296	GAGCUUGUA	CAGAAAUA	2297	526	UCGGACCG
CUGAUGAGGCCGAAAGGCCGAA	AUUUCUGU	2298	ACAGAAAUA	CGGUCCGA	2299	531	ACGUUUCG
CUGAUGAGGCCGAAAGGCCGAA	ACCGUAUU	2300	AAUACGGUC	CGAAACGU	2301	540	AACAGACC
CUGAUGAGGCCGAAAGGCCGAA	ACGUUUCG	2302	CGAAACGUU	GGUCUGUU	2303	544	CAAUAACA
CUGAUGAGGCCGAAAGGCCGAA	ACCAACGU	2304	ACGUUGGUC	UGUUAUUG	2305	548	UUGGCAAU
CUGAUGAGGCCGAAAGGCCGAA	ACAGACCA	2306	UGGUCUGUU	AUUGCCAA	2307	549	CUUGGCAA
CUGAUGAGGCCGAAAGGCCGAA	AACAGACC	2308	GGUCUGUUA	UUGCCAAG	2309	551	UGCUUGGC
CUGAUGAGGCCGAAAGGCCGAA	AUAACAGA	2310	UCUGUUAUU	GCCAAGCA	2311	562	UCCCCUUU
CUGAUGAGGCCGAAAGGCCGAA	AGUGCUUG	2312	CAAGCACUU	AAAGGGGA	2313	563	CUCCCCUU
CUGAUGAGGCCGAAAGGCCGAA	AAGUGCUU	2314	AAGCACUUA	AAGGGGAG	2315	575	UGUUUUCC
CUGAUGAGGCCGAAAGGCCGAA	AUUCUCCC	2316	GGGAGAAUU	GGAAAACA	2317	588	CCUCUCCC
CUGAUGAGGCCGAAAGGCCGAA	ACAUUGUU	2318	AACAAUGUA	GGGAGAGG	2319	603	CAAGUGGU
CUGAUGAGGCCGAAAGGCCGAA	AUGCCACC	2320	GGUGGCAUA	ACCACUUG	2321	610	CUGGAUUC
CUGAUGAGGCCGAAAGGCCGAA	AGUGGUUA	2322	UAACCACUU	GAAUCCAG	2323	615	AACUUCUG
CUGAUGAGGCCGAAAGGCCGAA	AUUCAAGU	2324	ACUUGAAUC	CAGAAGUU	2325	623	GUUUUCUU
CUGAUGAGGCCGAAAGGCCGAA	ACUUCUGG	2326	CCAGAAGUU	AAGAAAAC	2327	624	GGUUUUCU
CUGAUGAGGCCGAAAGGCCGAA	AACUUCUG	2328	CAGAAGUUA	AGAAAACC	2329	634	CUGUCCAG
CUGAUGAGGCCGAAAGGCCGAA	AGGUUUUC	2330	GAAAACCUC	CUGGACAG	2331	659	UGGUAAAU
CUGAUGAGGCCGAAAGGCCGAA	AUUCUGUC	2332	GACAGAAUU	AUUUACCA	2333	660	CUGGUAAA
CUGAUGAGGCCGAAAGGCCGAA	AAUUCUGU	2334	ACAGAAUUA	UUUACCAG	2335	662	GCCUGGUA
CUGAUGAGGCCGAAAGGCCGAA	AUAAUUCU	2336	AGAAUUAUU	UACCAGGC	2337	663	UGCCUGGU
CUGAUGAGGCCGAAAGGCCGAA	AAUAAUUC	2338	GAAUUAUUU	ACCAGGCA	2339	664	GUGCCUGG
CUGAUGAGGCCGAAAGGCCGAA	AAAUAAUU	2340	AAUUAUUUA	CCAGGCAC	2341	704	AGCUUUGC
CUGAUGAGGCCGAAAGGCCGAA	AUUUCCGC	2342	GCGGAAAUC	GCAAAGCU	2343	713	CCAGGCAG
CUGAUGAGGCCGAAAGGCCGAA	AGCUUUGC	2344	GCAAAGCUA	CUGCCUGG	2345		

Detailed Description Paragraph Table (32):

TABLE XIX

Porcine c-myb (region B) Hammerhead Ribozyme and Target Sequence (30 bp; nt. 1386 start; Human numbering system) (REVISED) Posi- Seq. Seq. tion Ribozyme ID No. Substrate ID No. 1394

GUGUUUAA	CUGAUGAGGCCGAAAGGCCGAA	AAAGAAUC	2346	GAUUCUUUC	UUAAACAC	2347	1396 AAGUGUUU
CUGAUGAGGCCGAAAGGCCGAA	AGAAAGAA	2348	UUCUUUCUU	AAACACUU	2349	1397	GAAGUGUU
CUGAUGAGGCCGAAAGGCCGAA	AAGAAAGA	2350	UCUUUCUUA	AACACUUC	2351	1404	GUUAUUGG
CUGAUGAGGCCGAAAGGCCGAA	AGUGUUUA	2352	UAAACACUU	CCAAUAAC	2353	1405	GGUUAUUG
CUGAUGAGGCCGAAAGGCCGAA	AAGUGUUU	2354	AAACACUUC	CAAUAACC	2355	1410	UUCAUGGU
CUGAUGAGGCCGAAAGGCCGAA	AUUGGAAG	2356	CUUCCAAUA	ACCAUGAA	2357	1423	CCAAGUCU
CUGAUGAGGCCGAAAGGCCGAA	AGUUUUCA	2358	UGAAAACUU	AGACUUGG	2359	1424	UCCAAGUC
CUGAUGAGGCCGAAAGGCCGAA	AAGUUUUC	2360	GAAAACUUA	GACUUGGA	2361	1429	GCAUUUCC
CUGAUGAGGCCGAAAGGCCGAA	AGUCUAAG	2362	CUUAGACUU	GGAAAUGC	2363	1440	CGUUAAAG
CUGAUGAGGCCGAAAGGCCGAA	AGGCAUUU	2364	AAAUGCCUU	CUUUAACG	2365	1441	ACGUUAAA
CUGAUGAGGCCGAAAGGCCGAA	AAGGCAUU	2366	AAUGCCUUC	UUUAACGU	2367	1443	GGACGUUA
CUGAUGAGGCCGAAAGGCCGAA	AGAAGGCA	2368	UGCCUUCUU	UAACGUCC	2369	1444	UGGACGUU
CUGAUGAGGCCGAAAGGCCGAA	AAGAAGGC	2370	GCCUUCUUU	AACGUCCA	2371	1445	GUGGACGU
CUGAUGAGGCCGAAAGGCCGAA	AAAGAAGG	2372	CCUUCUUUA	ACGUCCAC	2373	1450	GAGGCGUG
CUGAUGAGGCCGAAAGGCCGAA	ACGUUAAA	2374	UUUAACGUC	CACGCCUC	2375	1458	ACCACUGA
CUGAUGAGGCCGAAAGGCCGAA	AGGCGUGG	2376	CCACGCCUC	UCAGUGGU	2377	1460	UGACCACU
CUGAUGAGGCCGAAAGGCCGAA	AGAGGCGU	2378	ACGCCUCUC	AGUGGUCA	2379	1467	CAAUUUGU
CUGAUGAGGCCGAAAGGCCGAA	ACCACUGA	2380	UCAGUGGUC	ACAAAUUG	2381	1474	UAACAGUC
CUGAUGAGGCCGAAAGGCCGAA	AUUUGUGA	2382	UCACAAAUU	GACUGUUA	2383	1481	GGUGUUGU
CUGAUGAGGCCGAAAGGCCGAA	ACAGUCAA	2384	UUGACUGUU	ACAACACC	2385	1482	UGGUGUUG
CUGAUGAGGCCGAAAGGCCGAA	AACAGUCA	2386	UGACUGUUA	CAACACCA	2387	1492	CUCUAUGA
CUGAUGAGGCCGAAAGGCCGAA	AUGGUGUU	2388	AACACCAUU	UCAUAGAG	2389	1493	UCUCUAUG

CUGAUGAGGCCGAAAGGCCGAA AAUGGUGU 2390 ACACCAUUU CAUAGAGA 2391 1494 GUCUCUAU
CUGAUGAGGCCGAAAGGCCGAA AAAUGGUG 2392 CACCAUUUC AUAGAGAC 2393 1497 CUGGUCUC
CUGAUGAGGCCGAAAGGCCGAA AUGAAAUG 2394 CAUUUCAUA GAGACCAG 2395 1530 AAAAUAUG
CUGAUGAGGCCGAAAGGCCGAA AUUUUCCU 2396 AGGAAAAUA CAUAUUUU 2397 1534 UUCAAAAA
CUGAUGAGGCCGAAAGGCCGAA AUGUAUUU 2398 AAAUACAUA UUUUUGAA 2399 1536 AGUUCAAA
CUGAUGAGGCCGAAAGGCCGAA AUAUGUAU 2400 AUACAUAUU UUUGAACU 2401 1537 GAGUUCAA
CUGAUGAGGCCGAAAGGCCGAA AAUAUGUA 2402 UACAUAUUU UUGAACUC 2403 1538 GGAGUUCA
CUGAUGAGGCCGAAAGGCCGAA AAAUAUGU 2404 ACAUAUUUU UGAACUCC 2405 1539

Detailed Description Paragraph Table (34):

TABLE XX

Porcine c-myb (region B) Hairpin Ribozyme and Target Sequence (308 bp; nt. 1386 start; Human numbering system) (REVISED) Seq. Seq. Posi- ID ID tion Hairpin Ribozyme No. Substrate No.

1504
UUUUCACA AGAA GGUCUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2458 GAGACCA GAC 2459AAAA
1594 CAUGUUUG AGAA GUGUAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2460 CUACACC GUU
2461CAUG 1613 AUUUCUUG AGAA GCGAGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2462 ACUCGCA
GCU 2463AAAU

Detailed Description Paragraph Table (35):

TABLE XXI

Porcine c-myb (region A) Hairpin Ribozyme and Target Sequence (266 bp; nt. 458 start; Human numbering system) (REVISED) Seq. Seq. Posi- ID ID tion RZ No. Substrate No.

528
ACGUUUCG AGAA GUAUUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2464 AAAUACG GUC 2465ACGU
690 UUCCGCCC AGAA GUUCCC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2466 GGGAACA GAU
2467GGAA

Detailed Description Paragraph Table (36):

TABLE XXII

Rat c-myb (Region A) Hammerhead Ribozyme and Target Sequence (282 bp; nt. 428 start; Human numbering system) (REVISED) Position HH Ribozyme Seq. ID No. Substrate Seq. ID No. 467

CCUUUGAU CUGAUGAGGCCGAAAGGCCGAA AGCUCAGG 2468 CCUGAGCUC AUCAAAGG 2469 470 GGACCUUU
CUGAUGAGGCCGAAAGGCCGAA AUGAGCUC 2470 GAGCUCAUC AAAGGUCC 2471 477 GGUCCAGG
CUGAUGAGGCCGAAAGGCCGAA ACCUUUGA 2472 UCAAAGGUC CCUGGACC 2473 498 CACUCUUU
CUGAUGAGGCCGAAAGGCCGAA AUCUUCUU 2474 AAGAAGAUC AAAGAGUG 2475 509 ACAAGCUC
CUGAUGAGGCCGAAAGGCCGAA AUCACUCU 2476 AGAGUGAUA GAGCUUGU 2477 515 UUCUGGAC
CUGAUGAGGCCGAAAGGCCGAA AGCUCUAU 2478 AUAGAGCUU GUCCAGAA 2479 518 UAUUUCUG
CUGAUGAGGCCGAAAGGCCGAA ACAAGCUC 2480 GAGCUUGUC CAGAAAUA 2481 526 UCGGACCG
CUGAUGAGGCCGAAAGGCCGAA AUUUCUGG 2482 CCAGAAAUA CGGUCCGA 2483 531 GCGCUUCG
CUGAUGAGGCCGAAAGGCCGAA ACCGUAUU 2484 AAUACGGUC CGAAGCGC 2485 544 CAAUAACA
CUGAUGAGGCCGAAAGGCCGAA ACCAGCGC 2486 GCGCUGGUC UGUUAUUG 2487 548 UUGGCAAU
CUGAUGAGGCCGAAAGGCCGAA ACAGACCA 2488 UGGUCUGUU AUUGCCAA 2489 549 CUUGGCAA
CUGAUGAGGCCGAAAGGCCGAA AACAGACC 2490 GGUCUGUUA UUGCCAAG 2491 551 UGCUUGGC
CUGAUGAGGCCGAAAGGCCGAA AUAACAGA 2492 UCUGUUAUU GCCAAGCA 2493 562 UCCCUUUU
CUGAUGAGGCCGAAAGGCCGAA AGUGCUUG 2494 CAAGCACUU AAAAGGGA 2495 563 CUCCCUUU
CUGAUGAGGCCGAAAGGCCGAA AAGUGCUU 2496 AAGCACUUA AAAGGGAG 2497 575 UGUUUUCC
CUGAUGAGGCCGAAAGGCCGAA AUUCUCCC 2498 GGGAGAAUU GGAAAACA 2499 588 CCUCUCCC
CUGAUGAGGCCGAAAGGCCGAA ACAUUGUU 2500 AACAAUGUC GGGAGAGG 2501 609 UGGAUUCA
CUGAUGAGGCCGAAAGGCCGAA AUGGUUGU 2502 ACAACCAUU UGAAUCCA 2503 610 CUGGAUUC
CUGAUGAGGCCGAAAGGCCGAA AAUGGUUG 2504 CAACCAUUU GAAUCCAG 2505 615 AACUUCUG
CUGAUGAGGCCGAAAGGCCGAA AUUCAAAU 2506 AUUUGAAUC CAGAAGUU 2507 623 GUUUUCUU
CUGAUGAGGCCGAAAGGCCGAA ACUUCUGG 2508 CCAGAAGUU AAGAAAAC 2509 624 GGUUUUCU
CUGAUGAGGCCGAAAGGCCGAA AACUUCUG 2510 CAGAAGUUA AGAAAACC 2511 634 CUGUCCAU
CUGAUGAGGCCGAAAGGCCGAA AGGUUUUC 2512 GAAAACCUC AUGGACAG 2513 659 UGAUAAAU
CUGAUGAGGCCGAAAGGCCGAA AUUCUGUC 2514 GACAGAAUC AUUUAUCA 2515 662 GCCUGAUA
CUGAUGAGGCCGAAAGGCCGAA AUGAUUCU 2516 AGAAUCAUU UAUCAGGC 2517 663 UGCCUGAU
CUGAUGAGGCCGAAAGGCCGAA AAUGAUUC 2518 GAAUCAUUU AUCAGGCA 2519 664 GUGCCUGA
CUGAUGAGGCCGAAAGGCCGAA AAAUGAUU 2520 AAUCAUUUA UCAGGCAC 2521 666 GUGUGCCU
CUGAUGAGGCCGAAAGGCCGAA AUAAAUGA 2522 UCAUUUAUC AGGCACAC 2523

Detailed Description Paragraph Table (37):
TABLE XXIII

(Region B) Hammerhead Ribozyme and Target Sequences (262 bp; nt. 1421 start; human numbering system) (REVISED) Rat c-myb

Posi- tion	Seq. ID No.	Ribozyme	Seq. ID No.	Substrate	Posi- tion	Seq. ID No.	Substrate
1429							
1430	2524	CUCGGGCUU AGAUACGC	2525	GCGUAUCU CUGAUGAGGCCGAAAGGCCGAA AGCCCGAG			GGCGUAUC
1434	2526	UCGGGCUUA GAUACGCC	2527	CUGAUGAGGCCGAAAGGCCGAA AAGCCCGA			AGUAGGCG
1440	2528	GCUUAGAUA CGCCUACU	2529	CUGAUGAGGCCGAAAGGCCGAA AUCUAAGC			GGGUAAAG
1443	2530	AUACGCCUA CUUUACCC	2531	CUGAUGAGGCCGAAAGGCCGAA AGGCGUAU			GGAGGGUA
1444	2532	CGCCUACUU UACCCUCC	2533	CUGAUGAGGCCGAAAGGCCGAA AGUAGGCG			UGGAGGGU
1445	2534	GCCUACUUU ACCCUCCA	2535	CUGAUGAGGCCGAAAGGCCGAA AAGUAGGC			GUGGAGGG
1450	2536	CCUACUUUA CCCUCCAC	2537	CUGAUGAGGCCGAAAGGCCGAA AAAGUAGG			GAGGCGUG
1458	2538	UUUACCCUC CACGCCUC	2539	CUGAUGAGGCCGAAAGGCCGAA AGGGUAAA			ACCAAUGA
1460	2540	CCACGCCUC UCAUUGGU	2541	CUGAUGAGGCCGAAAGGCCGAA AGGCGUGG			UGACCAAU
1463	2542	ACGCCUCUC AUUGGUCA	2543	CUGAUGAGGCCGAAAGGCCGAA AGAGGCGU			UUGUGACC
1467	2544	CCUCUCAUU GGUCACAA	2545	CUGAUGAGGCCGAAAGGCCGAA AUGAGAGG			CAGUUUGU
1485	2546	UCAUUGGUC ACAAACUG	2547	CUGAUGAGGCCGAAAGGCCGAA ACCAAUGA			GUCUCGGU
1509	2548	CACCGUGUC ACCGAGAC	2549	CUGAUGAGGCCGAAAGGCCGAA ACACGGUG			UUCCUUUU
1522	2550	UGAAAACUN AAAAGGAA	2551	CUGAUGAGGCCGAAAGGCCGAA AGUUUUCA			UAAAGAUN
1526	2552	GGAAAACUC NAUCUUUA	2553	CUGAUGAGGCCGAAAGGCCGAA AGUUUUCC			GUUCUAAA
1528	2554	AACUCNAUC UUUAGAAC	2555	CUGAUGAGGCCGAAAGGCCGAA AUNGAGUU			GAGUUCUA
1529	2556	CUCNAUCUU UAGAACUC	2557	CUGAUGAGGCCGAAAGGCCGAA AGAUNGAG			GGAGUUCU
1530	2558	UCNAUCUUU AGAACUCC	2559	CUGAUGAGGCCGAAAGGCCGAA AAGAUNGA			UGGAGUUC
1536	2560	CNAUCUUUA GAACUCCA	2561	CUGAUGAGGCCGAAAGGCCGAA AAAGAUNG			GAUAGCUG
1542	2562	UUAGAACUC CAGCUAUC	2563	CUGAUGAGGCCGAAAGGCCGAA AGUUCUAA			CCUUUUGA
1544	2564	CUCCAGCUA UCAAAAGG	2565	CUGAUGAGGCCGAAAGGCCGAA AGCUGGAG			NACCUUUU
1552	2566	CCAGCUAUC AAAAGGUN	2567	CUGAUGAGGCCGAAAGGCCGAA AUAGCUGG			CGAGGAUU
1556	2568	CAAAAGGUN AAUCCUCG	2569	CUGAUGAGGCCGAAAGGCCGAA ACCUUUUG			CUUUCGAG
1559	2570	AGGUNAAUC CUCGAAAG	2571	CUGAUGAGGCCGAAAGGCCGAA AUUNACCU			GAGCUUUC
1567	2572	UNAAUCCUC GAAAGCUC	2573	CUGAUGAGGCCGAAAGGCCGAA AGGAUUNA			UUCUGGGA
1569	2574	CGAAAGCUC UCCCAGAA	2575	CUGAUGAGGCCGAAAGGCCGAA AGCUUUCG			AGUUCUGG
1578	2576	AAAGCUCUC CCAGAACU	2577	CUGAUGAGGCCGAAAGGCCGAA AGAGCUUU			UGGUGUGG
1588	2578	CCAGAACUC CCACACCA	2579	CUGAUGAGGCCGAAAGGCCGAA AGUUCUGG			CAUGUUUG
1589	2580	CACACCAUU CAAACAUG	2581	CUGAUGAGGCCGAAAGGCCGAA AUGGUGUG			GCAUGUUU
1608	2582	ACACCAUUC AAACAUGC	2583	CUGAUGAGGCCGAAAGGCCGAA AAUGGUGU			AAUUUCUU
1616	2584	UGGCAGCUC AAGAAAUU	2585	CUGAUGAGGCCGAAAGGCCGAA AGCUGCCA			CCGUAUUU
1617	2586	CAAGAAAUU AAAUACGG	2587	CUGAUGAGGCCGAAAGGCCGAA AUUUCUUG			ACCGUAUU
1621	2588	AAGAAAUUA AAUACGGU	2589	CUGAUGAGGCCGAAAGGCCGAA AAUUUCUU			GGGGACCG
1626	2590	AAUUAAAUA CGGUCCCC	2591	CUGAUGAGGCCGAAAGGCCGAA AUUUAAUU			CUUCAGGG
1640	2592	AAUACGGUC CCCUGAAG	2593	CUGAUGAGGCCGAAAGGCCGAA ACCGUAUU			GUCUNAGG
1644	2594	AAGAUGCUA CCUNAGAC	2595	CUGAUGAGGCCGAAAGGCCGAA AGCAUCUU			GGGGGUCU
1654	2596	UGCUACCUN AGACCCCC	2597	CUGAUGAGGCCGAAAGGCCGAA AGGUAGCA			CUACAUNA
1656	2598	GACCCCCUN UNAUGUAG	2599	CUGAUGAGGCCGAAAGGCCGAA AGGGGGUC			NACUACAU
1661	2600	CCCCCUNUN AUGUAGUN	2601	CUGAUGAGGCCGAAAGGCCGAA ANAGGGGG			UNUNNNAC
1664	2602	UNUNAUGUA GUNNNANA	2603	CUGAUGAGGCCGAAAGGCCGAA ACAUNANA			AGGUNUNN
1673	2604	NAUGUAGUN NNANACCU	2605	CUGAUGAGGCCGAAAGGCCGAA ACUACAUN			ACAUCNUG
	2606	NNANACCUN CANGAUGU	2607	CUGAUGAGGCCGAAAGGCCGAA AGGUNUNN			

Detailed Description Paragraph Table (38):
TABLE XXIV

(Region A) Hairpin Ribozyme and Target Sequences (282 bp; nt. 428 start; human numbering system)(REVISED) Rat c-mby

Posi- tion	Seq. ID No.	Ribozyme	Seq. ID No.	Substrate	Posi- tion	Seq. ID No.	Substrate
528							
690	2608	AAAUACG GUC	2609	GCGCUUCG AGAA GUAUUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA			GCGC
2611	2610	GGAAACA GAU		UUCUGCCC AGAA GUUUCC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA			AGAA

Detailed Description Paragraph Table (39):
TABLE XXV

Rat c-mby (Region B) Hairpin Ribozyme and Target Sequences (262 bp; nt. 1421 start; human numbering system)(REVISED)

Posi- tion	Seq. ID No.	Ribozyme	Seq. ID No.	Substrate

1495
UUUUCACA AGAA GGUCUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2612 GAGACCA GAC 2613AAAA
1604 AUUUCUUG AGAA GCCAGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2614 CCUGGCA GCU
2615AAAU 1623 CUUCAGGG AGAA GUAUUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA 2616 AAAUACG
GUC 2617GAAG

Other Reference Publication (22):
Chowrira et al., "In Vitro and in Vivo Comparison of Hammerhead, Hairpin, and Hepatitis Delta Virus Self-Processing Ribozyme Cassettes," J. Biol. Chem. 269:25856-25864 (1994).

Other Reference Publication (49):
Hertel et al., "Numbering System for the Hammerhead," Nucleic Acids Res., 20:3252 (1992).

Other Reference Publication (50):
Higashiyama et al, "A Heparin-Binding Growth Factor Secreted by Macrophage-Like Cells That is Related to EFG," Science 251:936-939 (1991).

Other Reference Publication (65):
Mamone et al., "Design of Hammerhead Ribozymes Targeted to Sequences in HIV, HSV and the RAT ANF Gene," Abstract of Keystone, CO (May 27, 1992).

Other Reference Publication (79):
Pieken et al., "Kinetic Characterization of Ribonuclease-Resistant 2'-Modified Hammerhead Ribozymes," Science 253:314-317 (1991).

Other Reference Publication (103):
Sjolund et al., "Arterial Smooth Muscle Cells Express Platelet-Derived Growth Factor (PDGF) A Chain mRNA, Secrete a PDGF-Like Mitogen, and Bind Exogenous PDGF in a Phenotype-- and Growth State-Dependent Manner," J. Cell. Biol. 106:403-413 (1988).

Other Reference Publication (108):
Ten Dijke et al., "Recombinant Transforming Growth Factor Type Beta-3 Biological Activities and Receptor-Binding Properties in Isolated Bone Cells," Mol. Cell Biol. 10:4473-4479 (1990).

Other Reference Publication (115):
Usman et al., "Chemical modification of hammerhead ribozymes: activity and nuclease resistance," Nucleic Acids Symposium Series 31:163-164 (1994).

CLAIMS:

3. The enzymatic RNA molecule of claim 1, wherein said enzymatic RNA molecule specifically cleaves any of the RNA sequences defined as SEQ. ID. NOS. 2, 4-6, 9-16, 18-20, 22-45, 47-98, wherein said enzymatic RNA molecule is in a hammerhead motif.

4. The enzymatic RNA molecule of claim 1, wherein said enzymatic RNA molecule is in a hammerhead motif.

213. The enzymatic RNA molecule of claim 1, wherein the binding arms of said enzymatic RNA molecule contain sequences perfectly complementary to any of the RNA sequences defined as SEQ. ID. NOS. 1-98, 101-120, or 123-129, wherein said enzymatic RNA molecule is in a hammerhead motif.

214. The enzymatic RNA molecule of claim 1, wherein the the binding arms of said enzymatic RNA molecule contain sequences perfectly complementary to any of the RNA sequences defined as SEQ. ID. NOS. 1-8, wherein said enzymatic RNA molecule is in a hepatitis delta virus motif.

215. The enzymatic RNA molecule of claim 5, wherein the binding arms of said enzymatic RNA molecule contain sequences perfectly complementary to any of the RNA sequences defined as SEQ. ID. NOS. 99, 100, or 130-148, wherein said enzymatic RNA molecule is in a hairpin motif.